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NITROGEN EXCRETION IN ASCIDIACEA

I. EXCRETION OF AMMONIA AND TOTAL NON-PROTEIN NITROGEN

BY IVAN GOODBODY
*Department of Natural History, University of Aberdeen**

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INTRODUCTION

ERRATUM

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T. Weis-Fogh, on 'Tetanic force and shortening

Line 11: *for* 175 mM. Na$^+$, *re*
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manhattensis about 1 l./hr. Figures calculated from data in Jørgensen, 1952.) The work of Needham (1929, 1931) and Delaunay (1931, 1934) has shown that there is a general relationship between the main nitrogenous end product of metabolism and the habitat of the animal or its embryo. Animals which have an abundant supply of water tend to excrete their nitrogen chiefly as ammonia which diffuses readily into the surrounding medium. Ammonia, however, is very toxic and unless it can be rapidly removed must be converted into less toxic substances; hence, in animals with a reduced water circulation the main nitrogenous end product becomes either urea or uric acid. Uric acid excretion is typical of animals in which water conservation is of primary importance either in the embryo or in adult life; typical examples are insects, reptiles and birds. It is unlikely that an ascidian at any stage of its life should suffer from difficulties of water conservation.

In the light of these facts a re-investigation of nitrogen excretion in ascidians was undertaken in an attempt to clear up what at first sight is an anomalous situation. The investigation is divided into two parts; in the present paper it will be shown that ascidians are essentially the same as other marine animals and excrete considerable quantities of ammonia such as to suggest that their protein metabolism is ammonotelic. In a second paper the problem of purine metabolism will be discussed in relation to the nature of the renal concretions.

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* Present address: University College of the West Indies, Mona, Jamaica, B.W.I.

The results are based on a study of three different species: *Ciona intestinalis* (Linnaeus) representing a type in which storage vesicles are absent; *Ascidiella aspersa* (Muller) a species with numerous small renal vesicles; *Molgula manhattensis* (Dekay) a species with a single large renal vesicle. For simplicity these are referred to throughout the text by their generic names only.

MATERIAL AND METHODS

Live ascidians were obtained from three sources. The Marine Laboratory at Millport provided *Ciona* and *Ascidiella*, the journey by rail taking about 18 hr. The Dove Marine Laboratory at Cullercoats also supplied *Ciona* and *Ascidiella*; animals collected there in the morning reached Aberdeen the same evening. The Marine Laboratory at Plymouth supplied *Molgula*, the rail journey taking 36–48 hr.

On arrival animals were kept in their containers until a temperature equilibrium had been reached between their water and the aquarium water to which they were to be transferred. Experiments were commenced as early as possible so as to ensure that most animals still had plenty of food in their guts during the experiments. Ideally it would be better to keep the animals for some days before conducting experiments as handling and transport are liable to increase their metabolic rate (Jørgensen, 1952); however, under the available aquarium conditions the animals would have become short of food in this time.

As far as possible animals selected for experiments were of 50 mm. or more in length. As much as possible of the surface dirt and encrusting organisms was removed from the test, either by picking off or scrubbing very gently with a soft nylon tooth-brush. Specimens of *Ciona* and *Molgula* were usually fairly free from encrustations, but *Ascidiella* often had barnacles and hydroids attached to the test. After cleaning, the animals were placed in clean sea water for at least an hour before being transferred to the experimental dishes.

Arrangement of the experiments

Each animal was placed in a glass crystallizing dish with a measured volume of filtered sea water, usually 200 or 250 ml., and the dish was covered with a sheet of glass; the animals were left in these dishes for periods varying between 20 and 35 hr., after which they were removed and the water filtered to remove any faeces. Except in one series of experiments when starved animals were deliberately used, any experiments in which no faeces were passed were rejected as also were those in which the animals showed signs of becoming unhealthy. The latter was tested for by tapping the dish sharply to ensure that the siphon closing mechanism was working correctly.

Control experiments consisted of a similar volume of sea water in a similar dish but containing no animal. The water from these dishes was treated in exactly the same manner as that from the experimental dishes. All the dishes were placed on a bench away from direct sunlight and in a room with a reasonably constant temperature.

Deproteinization

This was effected with the zinc sulphate-barium hydroxide method described by Somogyi (1945). 1 ml. of each solution was used for every 50 ml. sea water; this was added 1 ml. at a time and alternately zinc-sulphate and barium hydroxide, with shaking between each addition. The precipitate was filtered off and the clear filtrate used for subsequent analysis.

Estimation of ammonia

Ammonia was estimated by combining distillation in Markham's apparatus (1942) with nesslerization, the coloured solutions being then compared in a Hilger Spekker. 5 ml., samples of the deproteinized solution were distilled, the distillate being made up to 20 ml. with glass distilled water. After nesslerization the resulting coloured solution was compared against a blank made by nesslerizing 20 ml. distilled water.

Estimation of total soluble non-protein nitrogen (NPN)

The method chosen was to evaporate an acidified sample of the water almost to complete dryness, and to carry out a macro-Kjeldahl digestion on this followed by a micro-distillation in Markham's apparatus; the ammonia in the distillate was then determined as before by nesslerization.

Limitations of the method

The method outlined for the determination of ammonia and total NPN is open to criticism on a number of points, but providing the limitations are realized it provides a sufficiently accurate relationship between ammonia and NPN for the purposes of this investigation. A control experiment on the recovery of urea-N suggests that an accuracy of 10% is all that may be expected.

Bacterial action may be considered as the most serious source of error, as it is not possible to measure its extent. Dresel & Moyle (1950) passed the sea water through a bacterial filter candle before using it in similar experiments; in the present case at any rate this would appear to be of little value as it is not possible to sterilize the outside of the animal, and bacteria are certain to be introduced from this source when the animal is placed in the water. In order to reduce this error as far as possible the ammonia determinations were made at the earliest possible opportunity, usually within 6 hr. of the end of the excretion period, and the solutions were kept in a refrigerator until the distillations were made.

Small amounts of nitrogen will be introduced with the deproteinizing fluids and with the potassium sulphate in the digestion, but as these are quantitatively measured in equal amount in experimental and control fluids they will not alter the result.

Patel & Sreenivasan (1948) have shown that in Kjeldahl digestions there is a loss of nitrogen during prolonged heating after clearance of the digest when selenium is used as a catalyst; the error amounts to about 5% and is not considered here as being serious.

Dry weights

During the course of experiments with *Ascidiella* a suitable drying oven was not available; tissues were dried overnight at 56° C (the highest temperature available) and then transferred to a vacuum desiccator and dried over concentrated sulphuric acid for 24 hr. In the case of *Ciona* and *Molgula* tissues were dried overnight at 100° C and cooled in a desiccator. The two sets of data are not strictly comparable but the error in using the first method may not be very great.

EXPERIMENTAL RESULTS

Table 1 summarizes the data collected for all three species of ascidian so as to show the relation between ammonia production and total soluble non-protein nitrogen excreted. Table 2 shows the detailed results of experiments on *Ascidiella*; comparable data were obtained for both the other species. It is at once apparent from these tables that not only is there a considerable quantity of soluble non-protein nitrogen excreted by these ascidians, but that it is largely composed of ammonia.

Table 1. *Ammonia nitrogen (AmN) expressed as a percentage of total non-protein nitrogen (NPN) excreted by three species of ascidians*

Species	No. expts.	$\frac{\text{AmN}}{\text{NPN}} \times 100$	S.D.
Ciona intestinalis	17	87	±17
Ascidiella aspersa	8	95	± 7
Molgula manhattensis	5	88	±10

Table 2. *Ammonia nitrogen (AmN) and total soluble non-protein nitrogen (NPN) excreted by* Ascidiella aspersa

Expt. no.	Duration in hr.	µg. AmN excreted	µg. NPN excreted	$\frac{\text{AmN}}{\text{NPN}} \times 100$
1	29	461	435	106
2	29	159	184	86
3	29	110	123	89
4	23	686	673	102
5	23	278	292	95
6	23	371	428	87
7	23	498	489	102
8	23	216	231	94
				Mean 95

In order that more precise comparison of nitrogen excretion may be made between the different ascidians and between them and other animals, the total soluble non-protein nitrogen has been expressed as µg. excreted per hour per gram of dry weight.

This relationship is summarized for all three species in Table 3, and an example of the detailed data is shown for *Ciona* in Table 4. In view of uncertainty about the precise nature and function of the ascidian test (which certainly contains living material, but is nevertheless a protective organ comparable to the lamellibranch shell) the data have been expressed in two ways: as a function of body weight without the test, and as a function of total body weight including the test.

Table 3. *Amount of soluble non-protein nitrogen (NPN) excreted per hour by three species of ascidian, expressed as a function of dry body weight and total dry weight (body + test)*

Species	No. expts.	μg. NPN/hr./gm. of dry body wt. (without test), mean value	S.D.	μg. NPN/hr./gm. of total dry wt. (body + test), mean value	S.D.
Ciona intestinalis (fed)	4	79	±8	35	±5
Ciona intestinalis (starved)	4	27	±6	13	±3
Ascidiella aspersa	6	60	±30	26	±13
Molgula manhattensis	3	40	±4·5	26	±2·5

Table 4. *Excretion of NPN by* Ciona intestinalis *expressed as a function of the dry weight of the animal*

Expt. no.	μg. NPN excreted/hr.	Dry wt. of body (gm.)	Dry wt. of body plus test (gm.)	μg. NPN excreted/hr./gm. of body wt.	μg. NPN excreted/hr./gm. of total wt. (body and test)	Temp. (° C.)
1	21	0·253	0·535	83	39	12
2	15	0·185	0·415	81	36	12
3	15	0·228	0·549	66	27	12
4	18	0·207	0·459	87	39	12
				Mean 79	35	

DISCUSSION

Throughout the literature that has been studied, no reference has been found to the possibility of ascidians excreting nitrogen as ammonia or any other soluble product. There is, however, one quite incidental reference which merits mention. Przylecki (1926) in the course of experiments on uric acid metabolism in *Ascidia mentula*, injected them with uric acid and tested the water in which they were kept in an effort to identify the products resulting from the breakdown of this substance. In four experiments he found the ammonia content of the water to rise after 24 hr. by 0·05, 0·1, 0·2 and 0·3 unit. The units of measurement are not specifically mentioned, but in other parts of the same paper he uses milligrams as his unit, and it is presumably the same for these figures. If this is the case then the amount of ammonia excreted is of the same order as the quantities found in the present investigation, and may in fact be due to the normal metabolism of the animal and not to the injected uric acid.

The figures obtained for ammonia and total nitrogen excretion in ascidians may be compared with those for other animals in two ways. The ammonia nitrogen as a percentage of NPN may be compared in animals from a similar environment, and the total nitrogen excreted per gram of dry weight per hour may also be compared.

Table 5. *The percentage of ammonia nitrogen (AmN) excreted in relation to total non-protein nitrogen (NPN) in some marine animals*

Animal	$\frac{\text{AmN}}{\text{NPN}} \times 100$	Authority
Coelenterata		
Actinia	52·7	Delaunay (1934)
Annelida		
Aphrodite	80	Delaunay (1927)
Sipunculus	50	Delaunay (1934)
Arthropoda		
Gammarus locusta	80	Dresel & Moyle (1950)
Carcinus	67·8	Delaunay (1934)
Maia	42·9	Delaunay (1927)
Mollusca		
Mytilus	0–11·4	Spitzer (1937)
Mya	21·5	Delaunay (1927)
Littorina (summer)	40	
Littorina (winter)	61	Spitzer (1937)
Sepia	67	Delaunay (1934)
Octopus	33–50	Delaunay (1934)
Echinodermata	25–48	Delaunay (1934)
Urochordata		
Ciona intestinalis	87	Present work
Ascidiella aspersa	95	Present work
Molgula manhattensis	88	Present work

Table 5 shows the percentage of ammonia in relation to the total NPN in a number of marine animals. It is apparent that the figures found for ascidians are high compared with most other animals, the only ones that approach anywhere near to the ascidian figures being *Gammarus locusta* (80%) and *Aphrodite* (80%). Ammonia production varies with a number of different factors; Specht (1934) found that in the protozoan *Spirostomum* large amounts of ammonia were excreted when the oxygen tension was less than that corresponding to equilibrium with air. Oxygen tension is unlikely to have been low in the experiments of this investigation. More important perhaps is the relation between food and ammonia production. Thus in *Paramecium* Cunningham and Kirk (1941) found that in the absence of substrate ammonia production was about 91%, with fibrin it was about 76%, with glucose 35% and it disappeared altogether when starch was used as substrate. In *Lumbricus* Cohen & Lewis (1949) give a figure of 66–91% for normal animals and less than 10% when they are starving; for the same animal Delaunay (1934) gives 21–47% for starved and 47% for fed animals. In the present experiments with *Ciona intestinalis* values ranging from 47 to 98% ammonia nitrogen were obtained for starving animals and 61–100% for well-fed animals.

Some animals excrete more of their nitrogen as ammonia in winter than in summer. Thus *Littorina* (Spitzer, 1937) excretes 61 % as ammonia in winter and only 40 % in summer, and similarly *Unio* (*idem*) excretes 44 % in winter as against 25 % in summer; these differences are possibly due to differences in habitat and feeding at different seasons. The experiments on ascidians were all carried out in the winter months.

In the absence of evidence to the contrary it is considered that the high ammonia fraction in ascidians is normal and is correlated with the high turnover of water in these animals. Nevertheless, in the lamellibranch molluscs *Unio* and *Mytilus* which by virtue of their filter-feeding habits have a somewhat similar mode of life to ascidians, there is a relatively low ammonia fraction in the total excreted soluble nitrogen (Spitzer, 1937). However, both these animals have a relatively high amino-fraction in their excreted nitrogen which suggests that their protein metabolism is inefficient.

The figures calculated for total NPN in relation to dry weight of tissue in ascidians compare quite favourably with what is to be found in other animals. Very few actual figures are to be found in the literature, but they may be roughly calculated from the known data, so as to demonstrate that the excretion of nitrogen by ascidians is of an order comparable to that found in other animals. Data are given for milligrams of total nitrogen excreted per 24 hr. per 100 g. of wet weight for molluscs (Spitzer, 1937), *Anodonta* sp. and *Hirudo medicinalis* (Przylecki, 1922) and the earthworm *Pheretima posthuma* (Bahl, 1945). For amphipods and isopods Dresel & Moyle (1950) give data expressed in milligrams per 10 gm. wet weight per 24 hr. Dry weight figures for these species are not given; Weinland (1918) quotes a figure of 9 % of the wet weight in *Anodonta* (without the shell), and in the absence of better data dry weight has been assumed to be 10 % of wet weight for all the animals named. This procedure is questionable as some species will undoubtedly have higher values, but it provides an approximate means of comparing the data. The data for all these animals are set out in Table 6. The figures for molluscs from Spitzer and Przylecki are weights without shell.

The figures given for ascidians, while of the same order as for other animals, are somewhat high particularly when compared with the filter-feeding molluscs *Unio*, *Anodonta* and *Mytilus*; the discrepancy is not quite so big if the test is included in the ascidian weights. Perhaps a more important factor is the effect of transportation and handling on the ascidians; Jørgensen (1952) has shown that handling may cause an increase in the metabolic rate of *Molgula manhattensis* and that it takes several days for it to return to normal. In the present experiments a choice has had to be made between using animals which may be starving and animals which have recently been subjected to a lot of handling, and the latter have been used. In Jørgensen's experiments with *M. manhattensis* the metabolic rate appears to have almost doubled due to handling—the initial oxygen uptake was about 2·0 ml./hr. for fifteen animals and after 6–7 days had levelled out at about 0·7 ml./hr. If the figures for ascidians in Table 6 are halved they come very much more into line with those for the molluscan filter feeders.

In comparison with the amphipods, particularly *Gammarus zaddachi*, the ascidian values are low. This is to be expected if only on the grounds that amphipods are fairly active animals and will undoubtedly have a higher metabolic rate than ascidians; the difference may also be accentuated by the manner in which dry weight values have been estimated.

Table 6. *Comparison of total soluble non-protein nitrogen excreted in various animals expressed as a function of wet weight and of dry weight*

Notes. (i) Except in ascidians dry weights are not known; dry weight is taken as being 10 % of wet weight (see text). (ii) Season of the year is only known for ascidians and molluscs other than *Anodonta*. (iii) Species nomenclature of amphipods and isopods (other than *Asellus aquaticus*) follows Spooner (1947), Sexton and Spooner (1940), and Webb & Sillem (1906). *Asellus aquaticus* L. is presumably the species referred to.

Animal	Mg. N/100 g. wet wt./24 hr.		μg. N/g. dry wt./hr.		Authority
	Summer	Winter	Summer	Winter	
Ciona (without test)	—	—	—	79	Present work
Ciona (with test)	—	—	—	35	Present work
Ascidiella (without test)	—	—	—	60	Present work
Ascidiella (with test)	—	—	—	26	Present work
Molgula (without test)	—	—	—	40	Present work
Molgula (with test)	—	—	—	26	Present work
Unio	5·8	1·4	24	6	Spitzer (1937)
Mytilus	—	2·8	—	12	Spitzer (1937)
Paludina	2·7	1·4	11	6	Spitzer (1937)
Littorina	57·0	23·7	240	99	Spitzer (1937)
Limnaea	15·9	5·1	66	21	Spitzer (1937)
Arion	26·5	—	110	—	Spitzer (1937)
Anodonta	2·4		10		Przylecki (1922)
Hirudo	13·2		55		Przylecki (1922)
Pheretima	5·2		22		Bahl (1945)
Ligia oceanica	13		54		Dresel & Moyle (1950)
Oniscus asellus	3		12		Dresel & Moyle (1950)
Porcellio laevis	3		12		Dresel & Moyle (1950)
Armadillidium vulgare	4		17		Dresel & Moyle (1950)
Asellus aquaticus	26		108		Dresel & Moyle (1950)
Gammarus locusta	49		204		Dresel & Moyle (1950)
G. zaddachi	60		250		Dresel & Moyle (1950)
G. pulex	23		96		Dresel & Moyle (1950)
Marinogammarus marinus	11		46		Dresel & Moyle (1950)
M. pirloti	29		121		Dresel & Moyle (1950)
Orchestia sp.	20		83		Dresel & Moyle (1950)

The important point therefore, which emerges from this work is that ascidians are essentially similar to other aquatic animals in this aspect of their physiology, and there are good grounds for supposing that their protein metabolism is ammonotelic. The question of storage excretion and its significance will be returned to in a second paper.

SUMMARY

1. Ascidians have hitherto been regarded as eliminating their nitrogen in an insoluble form and storing the products either in special vesicles or in blood cells termed nephrocytes.

2. Three species of ascidian have been studied: *Ciona intestinalis*, *Ascidiella aspersa* and *Molgula manhattensis*. Evidence is brought forward to show that these ascidians excrete soluble nitrogen chiefly in the form of ammonia.

3. The quantities of soluble non-protein nitrogen excreted are slightly higher than is found in the filter-feeding molluscs; this is probably due in part to an increased metabolic rate subsequent to transport and handling.

4. Ammonia may form as much as 95% of the total non-protein nitrogen excreted.

5. The methods employed are subject to an error up to 10%.

Appropriate acknowledgements will be made in the second paper.

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THE CO-ORDINATION OF INSECT MOVEMENTS

II. THE EFFECT OF LIMB AMPUTATION AND THE CUTTING OF COMMISSURES IN THE COCKROACH (*BLATTA ORIENTALIS*)

By G. M. HUGHES

Department of Zoology, University of Cambridge

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INTRODUCTION

The normal rhythms of walking in the cockroach and other insects have been described in a previous paper (Hughes, 1952*a*). It was shown that movements of the six legs are co-ordinated in a very precise way and that two basic rules are obeyed in the insects studied. Alterations in this pattern can be readily detected and hence provide good material for a study of the role of the central and peripheral nervous systems in the co-ordination of locomotory movements. In the present paper experiments are described in which the removal of legs or the severing of thoracic commissures directly or indirectly prevents sensory impulses from reaching parts of the central nervous system. Removal of a leg also results in the remaining legs having to function under altered conditions. The modifications in walking so produced provide useful information about the mechanism of normal co-ordination.

A great deal of work has already been done in this field, but few investigators have made use of cinematography to analyse the effects of operations. For this reason they have usually failed to detect alterations in the locomotory movements which occur when relatively small parts of the neuromuscular mechanism have been removed. Detailed analyses are given from films of cockroaches with a single leg or any two legs removed. These have revealed certain deficiencies in earlier accounts of alterations in rhythm and have emphasized significant changes in posture of the legs. It is the purpose of this paper to draw attention to some of these and to show how they are related to the altered mechanical conditions and to the normal reflex mechanism of the leg.

MATERIALS AND METHODS

The cockroach used throughout these experiments was *Blatta orientalis*. Females were preferred because the absence of wings made their leg movements more visible and also facilitated the fixation of steel wires, small weights, etc., to the thorax. The normal walking of most insects was filmed before any experiment was performed but only slight individual variations were found. A Sinclair ciné camera was used at speeds of 24–32 frames/sec., with lighting provided by two

Photofloods or two 100 W. lamps and a spotlight. The insects walked at room temperature (18–20° C.) over a card marked with a grid.

The films were analysed by superimposing the body outline in successive frames and plotting the positions of the limb tips. In this way the position and length of the stride with respect to the body was obtained, and this gives a useful indication of the altered posture of the legs. As the results of this method were not published for the normal insect, examples from two different insects are shown in Fig. 1. The first is from the same shot as the sequence of stills in plate 12 (Hughes, 1952*a*),* while the second is from the insect whose middle legs were later amputated in the present work (Fig. 2).

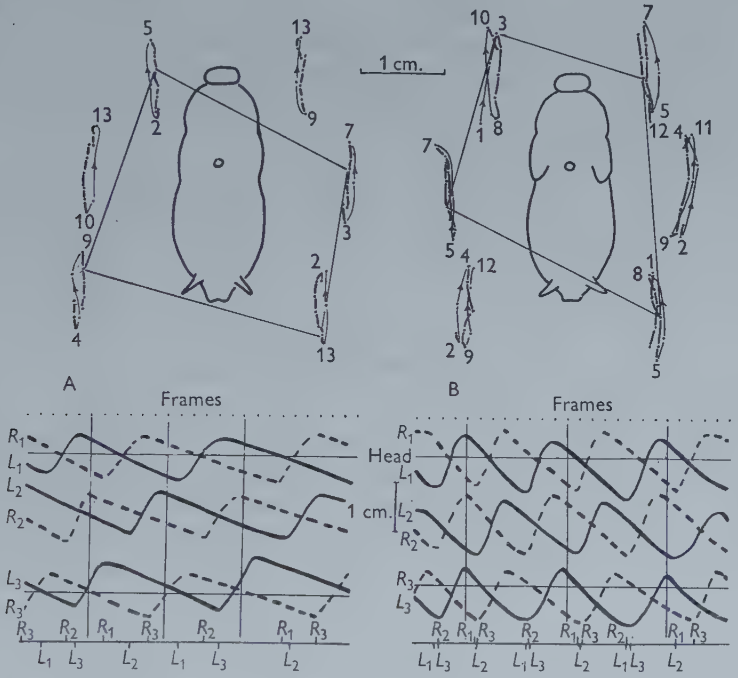

Fig. 1. Analyses from ciné films of the posture and rhythm of leg movements in two cockroaches during normal walking. In A the position of the limb tips relative to the body are only shown for alternate frames. The position of the centre of gravity is indicated by a circle and the area of support drawn for frame 11 in A and frame 3 in B. For more detailed explanation see text.

Successive positions of each limb tip are marked and when joined together they give the course of that limb tip relative to the body. Retraction is shown by a heavier line, and of course during this part of the cycle the limb is fixed relative to the ground. Only the frames at which a given leg is maximally protracted or retracted are numbered, but from these the position of the limb tips for any other frame can be determined. Then, by joining together the points for the different legs

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* In Text-fig. 8 of that paper the legends to A and B are incorrect and should be reversed as the area of support in an insect with the normal rhythm is shown in Text-fig. 8B and not 8A.

on the same frame, it is possible to construct the areas of support throughout the whole cycle and to see their relationship to the centre of gravity, which is usually situated about the level of the first abdominal segment. This has been done for frame 3 in Fig 1 B and it can be seen that during this phase the body is supported by L_1, R_1, L_2, R_3.

The tracings below each of these diagrams were obtained from the same strips of film, but here the position of each limb tip is plotted relative to the antero-posterior axis of the body. The points for each frame are plotted on vertical lines which are separated by a standard distance in order to introduce the time co-ordinate. The individual points for a given leg are omitted, but when joined up they form the curves as shown. The thick parts of the curves indicate the time when the leg is fixed on the ground and dashed lines are used for right legs. The points correspond to the intersection of the curves and lines drawn vertically for each frame. Three such lines are drawn, the interval between them being 0·25 sec. The two horizontal lines show the position of head and tip of the abdomen. The order of lifting is given at the bottom of each figure.

The analyses presented were selected to show the most typical movements. There are certainly variations from the patterns shown between both individuals and different runs of the same individual, but space does not permit a detailed description of these.

RESULTS

A. *Amputation experiments*

Carlet (1888) was the first worker to observe the changed rhythm of movements when the two middle legs of an insect are amputated. Later, Buddenbrock (1921) made a systematic series of experiments using the stick insect, *Dixippus*, which was excellent material for this purpose because of its slow speed. He found no perceptible change when a single leg was removed but consistent alterations in the rhythm followed the amputation of two legs. In all the latter cases diagonal legs moved almost simultaneously, the most extreme alteration occurring when the two middle legs were amputated. In such an insect the fore- and hindlegs of the same side now alternated whereas they had moved simultaneously in the intact insect. Bethe & Woitas (1930) confirmed these observations in their work on the walking of *Geotrupes*, *Dytiscus* and *Hydrophilus*. In the locust, ten Cate (1936) drew attention to the distinct delay between movement of a foreleg and the contralateral hindleg following amputation of the two middle legs. He also observed this tetrapod rhythm in the cockroach, *Periplaneta americana* (ten Cate, 1941). About the same time as most of the experiments described below were being carried out, similar amputation experiments on *Blatta* were done by Estartús & Ponz (1951). They did not use cinematography and their observations agree substantially with those of Buddenbrock.

In the present work, the legs were amputated at the coxo-trochanteral joint and the insects photographed immediately after the operation. They were then allowed to walk about for at least 3 days before the same insects were filmed again. No significant difference could be detected between these two sets of photographs.

Twelve different combinations of leg amputations were carried out, but most attention was directed towards the amputation of middle legs, as it is in this case that previous workers have thought the most marked changes occur. The principles observed in these experiments are applicable to other amputations which are described later and in less detail.

(1) *Amputation of a single middle leg* (R_2)

Apart from a tendency for the body axis to be inclined with the head slightly to the left of the direction of movement, these insects appeared to walk normally. However, the following modifications were found in analyses of photographs and tracings of leg movements of cockroaches from which the right middle leg had been removed (Figs. 2A, 3).

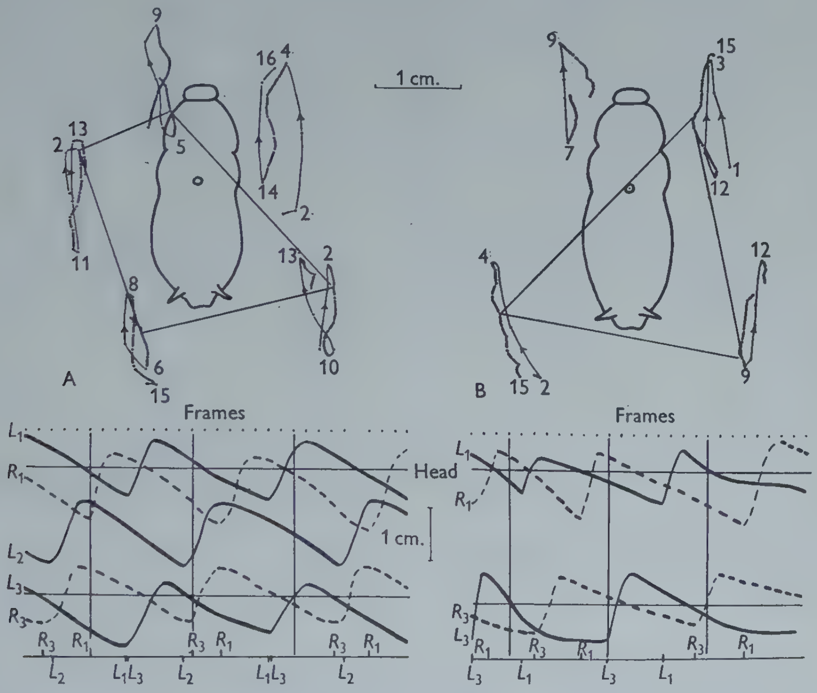

Fig. 2. Analyses of the leg movements of the insect shown in Fig. 1B after removal of the right middle leg (A) and later of the left middle leg also (B). The conventions are the same as in Fig. 1. The area of support is given for frame 3 in A and frame 8 in B.

(i) The right foreleg is retracted much further relative to the head before it is protracted.

(ii) The right hindleg is protracted much further forward and not retracted so far back relative to the head.

(iii) The legs on the right side are extended more lateral to the body than normally, while the left legs are held closer to the body axis. Both of these features are most marked in the hindlegs. On the injured side, these modifications will produce an increase in the lateral component of the strut effect which will help to compensate for the loss of the middle leg, which is normally very important in the maintenance of equilibrium about the longitudinal axis of the body.

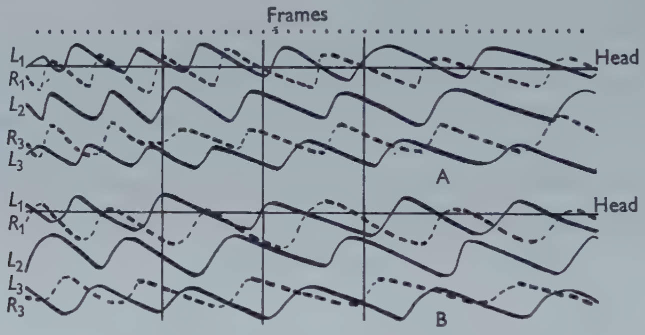

Fig. 3. Analyses of the movements of the legs with respect to the head in the same insect as shown in Fig. 2A but at two faster speeds.

Table 1

Speed (cm./sec.)	Protraction time / Retraction time	Stride length (cm.)				
		Fore		Middle	Hind	
Normal						
3·2	0·31	1·07		1·11	0·95	
5·0	0·35	1·06		1·07	0·95	
9·3	0·5	0·92		1·01	0·79	
R_2 amputated		L_1	R_1	L_2	L_3	R_3
3·0	0·23	0·71	1·21	0·97	0·83	1·07
5·0	0·31	0·71	1·01	0·89	0·74	0·99
6·0	0·34	0·92	0·97	0·97	0·77	0·8
L_2 and R_2 amputated						
2·6	0·17	0·93		—	1·15	
3·4	0·22	1·42		—	1·37	
4·3	0·28	1·11		—	1·35	

(iv) Table 1 shows that the stride length (distance moved by the limb tip relative to the head between complete retraction and protraction) is increased in both the hind- and forelegs on the side from which the middle leg has been removed. All figures in this table relate to the same insect.

(v) A constant feature is the change in timing of the two legs of the intact pro- and meta-thoracic pairs. R_1 tends to move later than normal with respect to L_1, whereas protraction of R_3 takes place earlier in the cycle of L_3 than normally (Fig. 4).

(vi) The rhythm of leg movements (Figs. 2A, 3) varies in detail and appears to depend on the speed of the insect. When moving slowly (2A) R_1 is protracted as soon as R_3 is in the supporting position. The three legs of the left side show the rhythm L_3, L_2, L_1. Protraction of L_1 is delayed until R_1 is placed on the ground. The same insect at a slightly faster speed (Fig. 3 B) uses an almost identical rhythm, but there is a distinct time lag between the instants at which R_3 obtains its *point d'appui* and when R_1 is protracted. This results in the two right legs alternating at nearly equal intervals of time after one another. At a still greater speed (Fig. 3 A), the insect uses the rhythm of a normal insect, but protraction of R_3 is usually delayed until R_1 is on the ground. There are occasional cases when both R_1 and R_3 are being protracted and there is no apparent support on the right side. This is not true, however, as R_3 is not lifted completely off the ground during protraction, but the body is displaced to the right because of the absence of any lateral thrust on the body by a leg on that side. While such differences in rhythm are found at different speeds it should be noticed that each rhythm remains fairly constant for a given run of at least 5 sec.

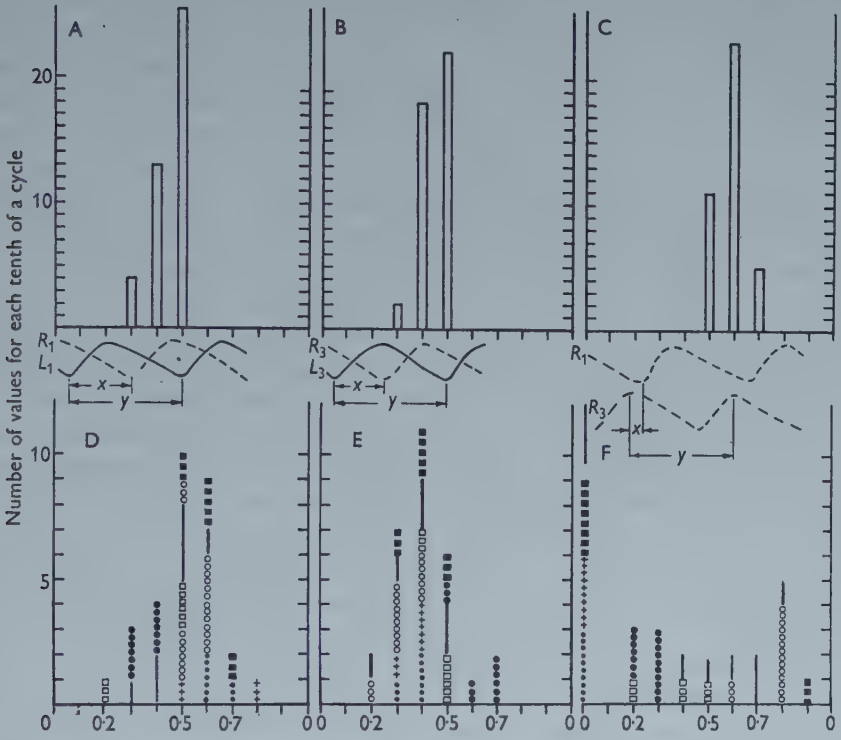

Fig. 4. Histograms to show variations in the timing of legs in a normal cockroach (A, B and C) and the same insect with the right middle leg removed (D, E and F). Values for x/y were determined from analyses such as in Fig. 3, as indicated by a diagram for each vertical pair. Groups of values are plotted for each tenth of a cycle. Note that in A, B and C the scale for the number of values plotted is twice that in D, E and F. A and D show the timing of the forelegs, B and E the hindlegs, and relationships between the fore- and hindleg of the right side are shown in C and F. In A, B and C variations in the ratio for different runs are not shown, but in D, E and F a different symbol is used for each of the seven runs analysed.

A more quantitative analysis of the different rhythms was obtained by considering the movements of the foreleg on the injured side in relation to the activity of the other foreleg and the ipsilateral hindleg. Normally R_1 alternates with L_1 so that protraction of R_1 occurs half-way between protractor movements of L_1. Thus in Fig. 4A protraction of R_1 occurs between values of 0·4 and 0·6 (mean = 0·475) in the ratio x/y. Similar values were also found for the hindlegs (Fig. 4B). Also in the intact insect we have the rule that R_1 protracts soon after R_2 is placed on the ground. In the absence of R_2, the movement of R_1 is modified and it is interesting to see how its activity is related to these two features of the normal co-ordination. From the curves showing the leg movements relative to the head it was possible to determine the instant at which R_1 was lifted with respect to (*a*) the interval between successive protractions of L_1, and (*b*) the interval between two instants at which R_3 is placed on the ground. The time of protraction of R_3 with respect to L_3 was likewise investigated. The measurements were made on seven different shots of the same insect running at different speeds along a straight path. The results (Fig. 4D–F) show that for a given run the phase relationship remains relatively constant but that there is some variation between different runs. The co-ordination of R_1 with L_1 is more constant than with R_3 which in turn is more co-ordinated with L_3. It can be seen that R_1 tends to be protracted later than half-way (i.e. $x/y \geq 0{\cdot}5$) through the cycle of L_1 in twenty-two of the thirty measurements. In the normal insect R_2 supports the right side of the body while R_1 protracts, and hence this delay in protraction of R_1 will help to compensate for the absence of R_2. The earlier protraction of R_3 ($x/y \leq 0{\cdot}5$) which is clear from the histograms would also have this effect.

The distribution of protraction of R_1 with respect to R_3 (Fig. 4F) shows a large peak between 0–0·1. These are cases where R_1 protracted within one-tenth of a cycle of R_3 being placed on the ground. The absence of protraction within the next tenth of a cycle is interesting in that it may represent the time during which R_2 is normally protracted. The even distribution between 0·4 and 0·7 covers the range found for the intact cockroach (Fig. 4C). The minor peak at 0·8 followed by only a single case in the next category, shows that protraction of R_3 is delayed until R_1 is on the ground, and that it is rare for R_1 to be lifted during the brief period when R_3 is off the ground. In half of the cases examined protraction of R_1 or R_3 took place almost immediately the other right leg was placed on the ground. It is clear that the segmental co-ordination of R_1 and L_1 (and of R_3 and L_3) is more constant than the co-ordination of R_1 and R_3, but in all three cases it is less rigid than in the normal insect.

Such changes which follow the amputation of a single middle leg are related to a loss of the support which it normally provides. Modifications in the movements of the fore- and hindleg on the injured side enable them to replace the amputated leg functionally. This is most apparent at slow speeds, but even at the fast speeds both the position of the *points d'appui* and the length of the stride are altered. Static stability throughout the cycle is maintained as in the intact animal, but is reduced if the insect moves very rapidly.

It is possible to interpret the changed phase relations of some of the legs in terms of the known segmental reflexes. Pringle (1940) has confirmed Rijlants (1932) finding that an increase in the resistance to depression of a leg produces augmentation of the electrical activity in the depressor muscles. During retraction of R_1 in a cockroach without R_2, the proportion of the body weight supported by R_1 tends to be greater than normal; its protraction relative to L_1 will be delayed, and consequently it will have a longer stride length and move further back relative to the head. At slower speeds, when static stability is rigidly maintained, R_1 does not protract until R_3 has taken up its supporting position. When this happens there will be a sudden decrease in the proportion of body weight supported by R_1 which will excite the levator reflex (Pringle, 1940). The way in which R_3 sometimes protracts as soon as R_1 is placed on the ground, suggests that the consequent decrease in its supporting function might also play a part in bringing about earlier protraction in this leg. It is also probable that some ipsilateral descending pathway is excited when R_1 is placed on the ground. These factors all contribute to the earlier protraction of R_3 relative to L_3. The alterations in timing of R_1 and R_3 relative to the contralateral leg of the same segment have functional advantages with respect to the transverse strut effects. It has been pointed out (Hughes, 1952*a*) that during retraction this component increases in a foreleg but decreases in a hindleg. Normally the contralateral middle leg antagonizes these effects, and in the absence of R_2, R_1 and R_3 take over this function. Later protraction of R_1 and earlier protraction of R_3 both have the result that their maximum transverse components will be exerted to oppose the action of L_1 and L_3. These increases in both the supporting function and lateral strut action of R_1 and R_3 probably produce an exaggerated 'rebound' of the levator and protractor muscles which will give an increased stride length.

Further evidence for this sort of interpretation is given by a cockroach which had R_2 less well developed than normally. The rhythm showed some of the features noted above, particularly (i), (ii) and (v).

(2) *Amputation of both middle legs* (R_2, R_2)

This operation had a much greater effect than the removal of only a single leg. The insects moved more slowly and there was a tendency for the body to touch the ground between the two remaining pairs of legs.

The limb movements are modified in the following ways:

(i) The stride length of all legs is increased, so that observations (i) and (ii) of the five-legged insect apply to both sides of the body. This increase in stride length is most noticeable in the hindlegs which are protracted much farther forward. Their *points d'appui* are more lateral to the body than in the normal insect.

(ii) The two legs of a segment are protracted at nearly equal intervals of time after one another.

(iii) The rhythm of leg movements is changed. The graphs (Fig. 2B) show that the order of protraction is R_3, R_1, L_3, L_1, R_3, but this was not invariable. This rhythm is identical with that seen in a walking tetrapod.

Previous workers have rightly emphasized the immediate nature of these changes, but at first there is a lack of precision about them which disappears after a few days.

This subsequent increase in efficiency is a subsidiary phenomenon, however, for the change in rhythm is instantaneous as is emphasized by the following observation.

If the two middle legs were amputated from a cockroach which was freely suspended from a steel wire attached to the thoracic tergites the remaining legs immediately performed active movements. Analysis of films of simultaneous side and dorsal views showed that the legs are often co-ordinated in the diagonal rhythm described above. The forelimbs were easily followed but the hindlimb movements were made up of the following phases:

(i) Levation and movements forwards.
(ii) Movement backwards.
(iii) Depression.
(iv) Flexion of the femoro-tibial joint.

The forelegs alternate normally and are related to movements of the hindlegs in that they commence to move forwards when the ipsilateral hindleg has completed phase (i). The rhythm of movements relative to the head is very similar to that described in the four-legged insect when walking on the ground. This co-ordination persists for about 10 sec., after which the movements become more spasmodic and apparently disco-ordinated. These experiments indicate some measure of intersegmental co-ordination in the absence of drag or changes in the distribution of weight.

One of the reasons for paying particular attention to the amputation of both middle legs was because previous workers maintained that the greatest alteration in rhythm followed this operation. This conclusion was based, however, on the view that the two tripods of support L_1, R_2, L_3 *and* R_1, L_2, R_3 were the basic units of the normal rhythm, so that L_1 and L_3 moved simultaneously in one phase and R_1 and R_3 in the other. More detailed study has shown (Hughes, 1952*a*) that, in the cockroach at least, the two sequences R_3, R_2, R_1 and L_3, L_2, L_1 are best considered as the basic units which overlap one another and only at the very fastest speeds does a foreleg move almost simultaneously with the hindleg on the same side. The four-legged cockroach only moves at speeds comparable with the slower speeds of a normal insect and hence is best considered in relation to these. It is obvious that the rhythm R_3, R_1, L_3, L_1, R_3 results when the two middle legs are omitted from the rhythm R_3, R_2, R_1, L_3, L_2, L_1, R_3 which is the basic rhythm at very slow speeds.

The new rhythm obeys the same rules as the rhythm in the intact insect. In fact we shall find that the rule that no leg is lifted before the leg behind has taken up its supporting position is obeyed in all the other amputation experiments. The only exceptions found were the few cases mentioned above when the insect moved rapidly following the removal of a single middle leg. A number of exceptions were found to the other rule after some of the amputations described below, the two legs of a segment sometimes moving together or very nearly so.

(3) *Amputation of a single foreleg* (R_1)

These insects appeared to move in a fairly normal way but the body axis was usually inclined with the head to the left of the direction of movement (Fig. 5A).

(i) R_2 and R_3 are protracted farther forwards than normally and have their stride lengths increased relative to the contralateral legs.

(ii) Legs on the right side, particularly R_3, are placed more laterally.

(iii) Left legs move nearer to the body axis, especially the foreleg which often retracts beneath the prothorax. Stride of L_1 is usually increased.

(iv) L_2 also quite often protracted far forwards and overlaps L_1. This is necessary to support the body when L_1 is lifted.

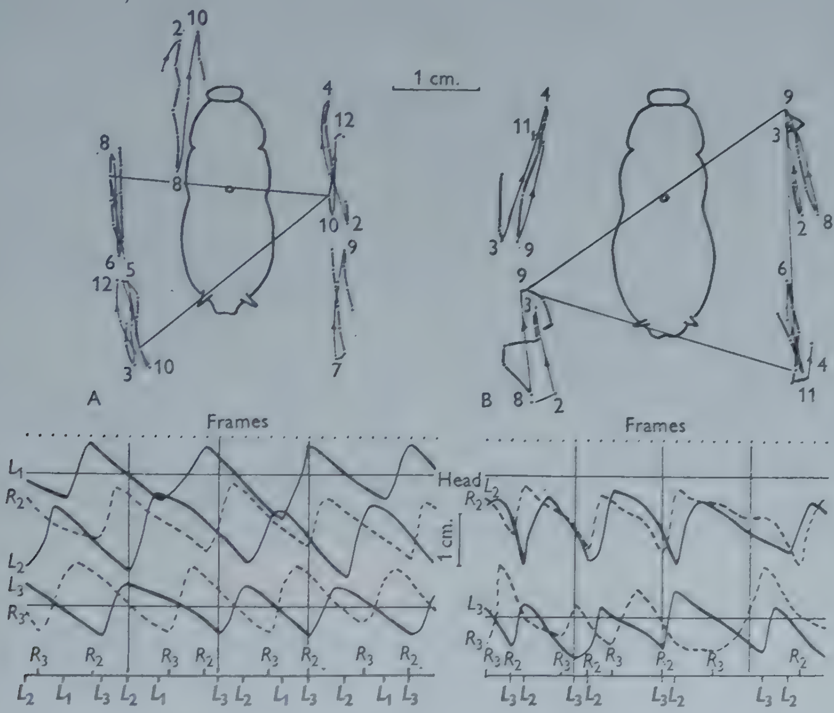

Fig. 5. Analyses of the leg movements of a cockroach after removal of (A) the right foreleg and (B) both forelegs. The area of support is drawn for frame 9 in both cases.

(v) The rhythm of leg movements retains the basic components L_3, L_2, L_1 and R_3, R_2 with a gap left for R_1. The precise timing of these two basic rhythms varies, for example, L_1 may move before or after R_3. There is a modification, however, in so far as R_2 does not protract until L_1 has been placed on the ground and consequently R_2 and L_3 usually move simultaneously. This persists at quite fast speeds when normally the three legs of the tripod L_1, R_2, L_3 would move very soon after one another. This modification together with the altered position of the intact foreleg ensures that there is adequate support at the anterior end of the body when R_2 is protracted. If the insect used the normal rhythm, as supposed by previous authors, there would be an unstable phase when both L_1 and R_2 were off the ground.

(4) *Amputation of both forelegs* (R_1, L_1)

These insects slipped on the ground a great deal but frequently showed a diagonal rhythm when walking slowly. More often the middle legs protracted together and the animal fell forwards. At fast speeds the diagonal legs moved synchronously and the head was pushed along on the ground (Fig. 5 B).

(i) The two middle legs have a slightly increased stride and they are both protracted farther forwards than in the normal insect.

(ii) The two hindlegs are also protracted farther forwards.

(iii) All four legs are spread out much farther from the body axis. This general impression is emphasized by the relatively large distance separating the strides of legs on the same side.

(iv) The rhythm is made up of the two basic components R_3, R_2 and L_3, L_2, but these may vary in their phasing with one another.

The two legs of a segment do not move at equal times after one another and in many cases the middle legs move almost simultaneously. As a result progression is jerky and the front end of the insect is alternately raised and lowered. In the prints this latter feature is very clear because the shadow cast from a light source behind the insect increases as both middle legs retract simultaneously and then decreases as first one and then the other protracts.

In some cases the diagonal rhythm R_2, L_3, L_2, R_3, R_2, L_3 is found but this is certainly not the most common. Protraction of the four legs is not evenly distributed throughout the cycle. This is even more marked at slower speeds. The importance of (i) and (ii) is well shown in Fig. 5 B. If R_2 and L_3 were not so far forwards when L_2 is protracting, the centre of gravity would fall outside the triangle of support as happens when the stride lengths shorten at faster speeds.

(5) *Amputation of a single hindleg* (R_3)

These insects were able to move very fast and seemed to be using the normal rhythm. However, the impression was given that L_3 was being dragged and that it exerted little propulsive thrust (Fig. 6 A).

(i) R_1 and R_2 are retracted farther back than normal.

(ii) Both middle legs tend to be more lateral to the body.

(iii) L_3 has a relatively short stride and is placed much nearer to the body axis.

(iv) The basic units of the rhythm, L_3, L_2, L_1 and R_2, R_1, are maintained. In the rhythm shown in Fig. 6 A, protraction of R_2 is delayed until L_3 has taken up its supporting position. This results in the two middle legs often being moved very soon after one another, if not simultaneously.

This modification to the rhythm is again adaptive since L_3 is able to support the hind end of the body while R_2 is protracted. If it did not occur there would be a phase of the cycle when only R_1, L_1 and L_2 were supporting the body. The altered position of L_3 ensures that the centre of gravity falls within the triangle of support R_1, L_1, L_3 as shown in Fig. 6 A.

As was emphasized when considering the amputation of a single middle leg the precise rhythm varies, and another interesting rhythm is shown in Fig. 8A. Here L_3 is not protracted until R_2 is on the ground. Thus instead of L_2 and R_2 moving together, L_1 and R_2 protract at the same time which is more like the normal rhythm. When R_2 is lifted it is interesting to see how L_3 pushes the body farther away from the *point d'appui*, presumably because of the sudden decrease in the tranverse strut effect of R_2.

(6) *Amputation of both hindlegs* (R_3, L_3)

Of all the four-legged cockroaches examined those with the two hindlegs removed were the least affected, in that they moved actively along a straight path and with no awkwardness about their limb posture and movement. However, they dragged the abdomen along the ground for most of the cycle. It is evident from Fig. 6B that during the protraction of a middle leg the centre of gravity falls well behind the area of support.

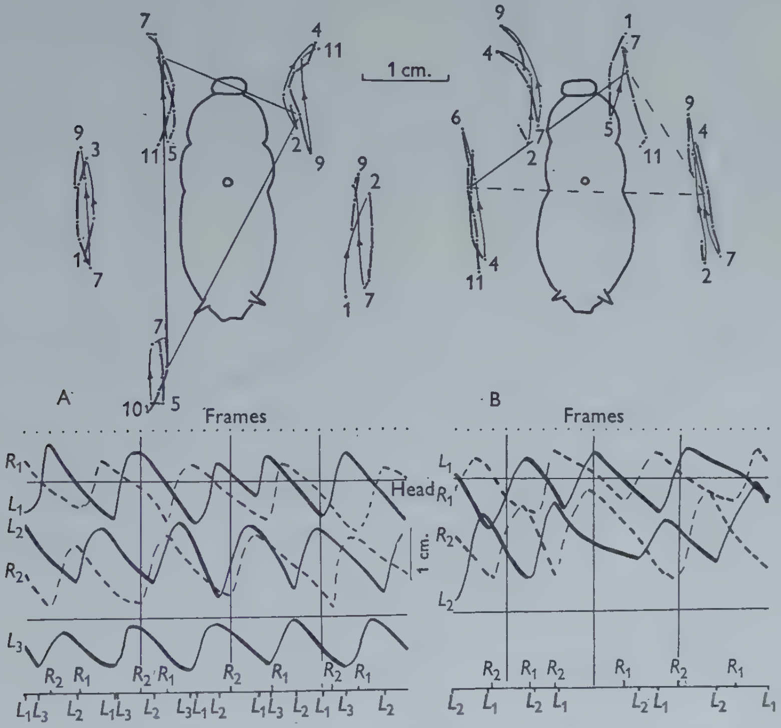

Fig. 6. Analyses of the leg movements in a cockroach after removal of (A) the right hindleg and (B) both hindlegs. The area of support is drawn for frame 8 in both cases.

(i) The stride length of all the legs is increased. This is especially true of the middle legs which are protracted farther forwards and retracted more than normally. Their position of maximum protraction is anterior to the position of the fully retracted forelegs.

(ii) The action of the forelegs tends to draw the body towards the *point d'appui* so that the path of the limb tip relative to the body is not parallel to the body axis. This feature is also found with only a single hindleg amputated, and probably indicates that the forelegs are exerting more of a pull than normally. The impression is given that both middle and forelegs exert more of a pulling than a pushing action, i.e. they seem to lever the body forwards.

(iii) The rhythm of the four legs is not always the diagonal rhythm R_1, L_2, L_1, R_2, R_1, etc., described by previous authors although this is certainly the most common, and the two sequences L_2, L_1, and R_2, R_1 are always present. At faster speeds it is usual for the two pairs of diagonal legs to alternate with one another as shown at the beginning of the rhythm in Fig. 6B.

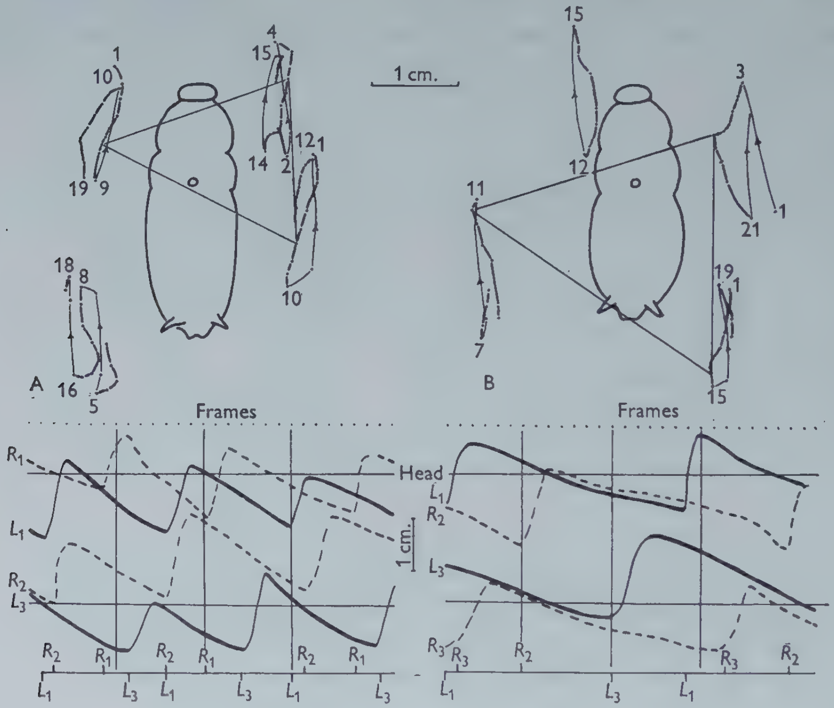

Fig. 7. Analyses of the leg movements of cockroaches following the removal of (A) the left middle and right hindlegs, and (B) the right fore and left middle legs. The area of support is drawn for frame 7 in A and frame 13 in B. In the latter only points for every other frame are shown.

(7) *Amputation of a foreleg and contralateral middle leg* (R_1, L_2).

Sometimes these insects did not seem to walk very well, the torsi being oriented abnormally with respect to the tibia. However, the legs were co-ordinated in a good diagonal rhythm (Fig. 7B).

(i) The stride length of L_1, L_3 and R_2 is increased.

(ii) R_2 and L_3 are protracted much farther forwards than in the normal insect.

(iii) The *point d'appui* of L_3 is much more lateral to the body axis, and R_3 moves closer to the body. During retraction L_1 is also held close to the body.

(iv) The typical rhythm is L_3, L_1, R_3, R_2, L_3.

It is rare for both pairs of diagonal legs to alternate with one another although quite often either L_1 and R_3, or R_2 and L_3 protract together at faster speeds giving the rhythms $L_3, \frac{L_1}{R_3}, R_2, L_3$ or $R_3, \frac{R_2}{L_3}, L_1, R_3$.

These animals combine the modifications shown by insects with a single foreleg and a single middle leg amputated. The importance of these modifications is that they again ensure that the centre of gravity falls within the triangle of support throughout the whole cycle. Thus at frame 13 the anterior border of the triangle of support would be behind the centre of gravity if L_3 had not been protracted so far forwards. It is also interesting to notice how the path of R_2 changes direction after this phase, suggesting that the leg begins to exert an extensor thrust along its axis in place of a tractive function which is taken over by L_1. There can be little doubt that R_2 is replacing R_1 functionally.

(8) *Amputation of a foreleg and contralateral hindleg* (L_1, R_3)

The tracing and rhythms shown in Fig. 8 are of the same insect before and after amputation of L_1 in addition to R_3. Even after this operation the insect moves quite rapidly, but a tendency was noticed for it to fall forwards as the middle legs protracted simultaneously.

(i) The most marked change takes place in the movement of L_2 which is protracted much farther forwards and has a greatly increased stride length.

(ii) L_3 does not move quite so close to the body axis.

(iii) R_1 and R_2 are more spread out and R_2 is not retracted so far as when only R_3 is amputated.

(iv) The rhythm is usually R_1, L_2, R_2, L_3, R_1, L_2, etc. This is obviously not the diagonal rhythm.

There are moments of instability when only two legs are definitely on the ground. This takes place when R_1 is protracted and the body is supported chiefly by the two middle legs which are being retracted almost synchronously. The shadow on the photographs showed that the anterior part of the body is lifted up during this phase, and that shortly afterwards it falls (the photograph is blurred) as R_1 is placed on the ground. Frame 5 in Fig. 8B shows this very well as in this case L_3 is not quite in the supporting portion as it usually is at this phase of the cycle. In the next frame (6) all the legs are on the ground and the insect extremely stable.

(9) *Amputation of a middle leg and contralateral hindleg* (L_2, R_3)

Except for some apparent dragging of L_3, these insects moved quite well and showed a good diagonal rhythm (Fig. 7A).

(i) L_1 is not protracted so far forwards and is retracted farther than normal. This leg is also more lateral to the body, particularly towards the end of retraction.

(ii) Both R_1 and R_2 are retracted farther than in the intact insect. The stride length of R_2 is increased and it plays an important part in supporting the hind end of the body while L_3 is being lifted forwards.

(iii) L_3 moves fairly normally but in some cases it is protracted a little farther forwards than normal. Immediately after the operation this leg was scarcely active.

(iv) The rhythm is nearly always R_2, R_1, L_3, L_1, R_2, etc., with the legs protracting at more or less equal intervals after one another. But quite often there is a tendency for a given pair of diagonal legs to move almost simultaneously, e.g. L_1 and R_2 in the rhythm illustrated. At other times the opposite diagonal pair are more synchronized.

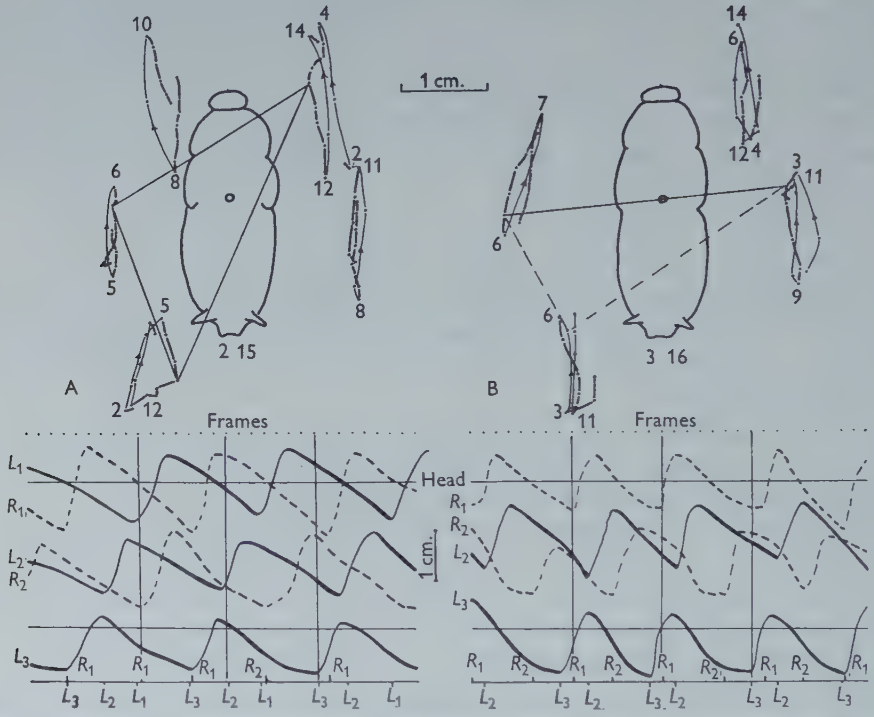

Fig. 8. Analyses of the leg movements in the same cockroach (A) following the removal of the right hindleg and (B) after amputation of the left foreleg also. Area of support is shown at frame 8 in A and frame 5 in B.

The significance of these changes can be seen at frames 6–7 when L_3 is being protracted. The centre of gravity only just comes within the triangle of support which would not happen unless features (i) and (ii) were present.

(10) *Amputation of a middle leg and the ipsilateral hindleg* (R_2, R_3)

In these insects only the foreleg supported the body on the right side, and consequently the tip of the abdomen tended to touch the ground on that side. This was particularly noticeable during protraction of R_1. When moving rapidly the body axis was inclined with the head to the right of the line of progression (Fig. 9A).

(i) Without ciné analyses it is clear that the right foreleg retracted farther backwards than normally, and that the fore- and hindlegs on the intact side move closer to the body axis. In some cases the action of the single hindleg (L_3) is almost directly behind the abdomen (Fig. 9A).

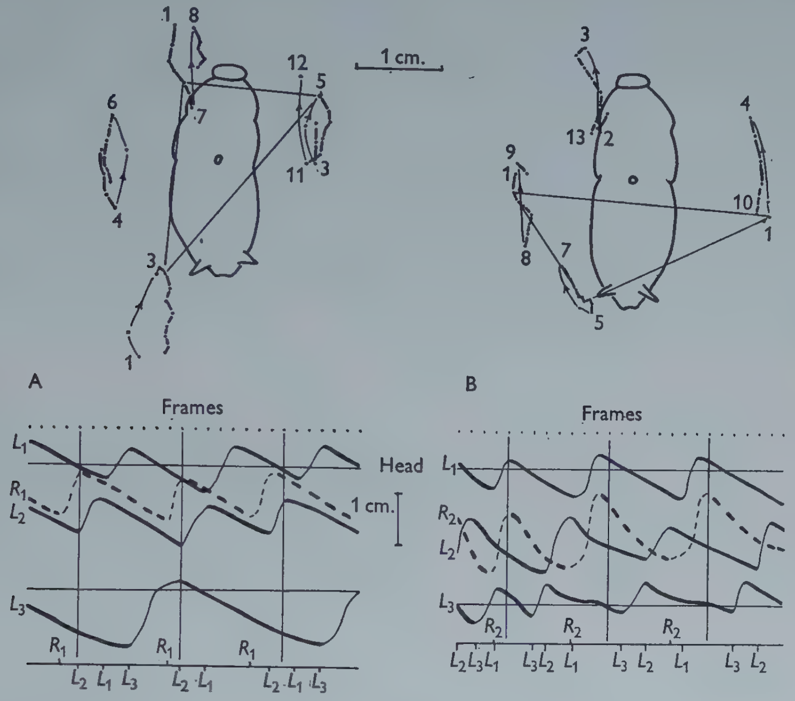

Fig. 9. Analyses of the leg movements in cockroaches following the removal of (A) the right middle and hindlegs, and (B) the right fore- and hindlegs. Areas of support are drawn for frames 5 (A) and 2 (B).

(ii) Protraction of R_1 is delayed so that it is not lifted until after half-way through the cycle of L_1. The stride of R_1 is generally increased.

(iii) Usually the sequence L_3, L_2, L_1 is shown, but at faster speeds L_3 sometimes moves at half the frequency of the other three legs.

(iv) L_2 usually protracts as soon as R_1 is placed on the ground, but sometimes it is lifted while R_1 is off the ground.

The posterior displacement of the strides of R_1 and L_3, together with their positions being respectively farther from and closer to the body axis, ensure that the line joining the *points d'appuis* of these two legs will pass behind the centre of gravity. This is particularly important shortly after these two legs are placed on the ground (Fig. 9A, frame 5).

(11) *Amputation of a foreleg and the ipsilateral hindleg* (R_1, R_3)

Immediately after these amputations the insect still moved rapidly and did not appear badly affected as it scuttled along. But at slower speeds the movement was much more jerky and it fell to the right during protraction of R_2. As in other insects with only a single leg on one side, the head was inclined to the injured side of the direction of movement. These insects tired rapidly and then moved in small clockwise circles because activity of the single right leg was greatly reduced (Fig. 9B).

(i) Posture of the legs is modified so that the three left legs are close to the body, whereas the right middle leg is extended laterally. In fact L_1 tends to move under the body during retraction and this makes it difficult to plot out its movements from the film.

(ii) The sequence L_3, L_2, L_1 is always present.

(iii) The timing of R_2 with respect to the other legs varies but it usually takes place shortly after L_2 is placed on the ground, and hence almost simultaneously with protraction of L_1. Thus the rhythm is usually either L_3, L_2, R_2, L_1, L_3 or L_3, L_2, L_1, R_2, L_3.

(iv) The centre of gravity falls within the area of support except for the phase just before and when R_2 is protracted.

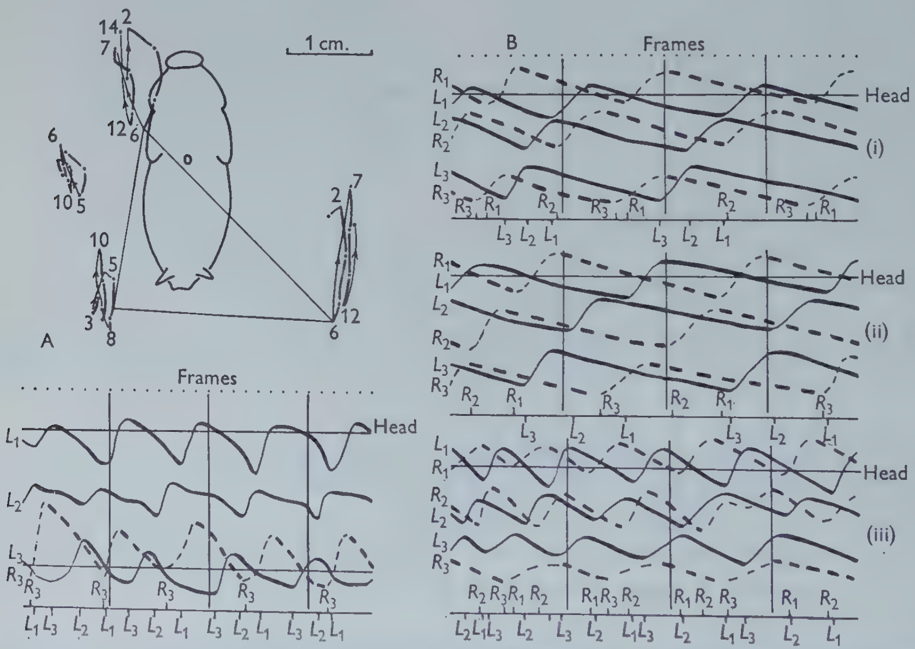

Fig. 10. (A) Analyses of the leg movements in a cockroach with the right fore- and middle legs amputated. The area of support at frame 6 is shown. (B) The rhythm of leg movements plotted relative to the head in cockroaches with the following commissures cut: (i) and (iii) right meso-metathoracic; (ii) right pro-mesothoracic.

(12) *Amputation of a foreleg and ipsilateral middle leg* (R_1, R_2)

Again these insects moved quite rapidly and the body axis was inclined with the head to the right of the line of progression. They tended to fall just before R_3 was protracted (Fig. 10A).

(i) R_3 was extended laterally and protracted farther forwards but did not retract so far as in the normal insect. The stride length of this leg was often greater than that of L_3.

(ii) All three legs on the left side were held closer to the body. In addition L_2 is protracted farther forwards than normally.

(iii) The sequence L_3, L_2, L_1 was well defined. R_3 often protracted simultaneously, or shortly before, L_1 giving the order of lifting L_3, L_2, R_3, L_1, L_3, etc.

Analyses of superimposed frames showed that R_3 protracted as soon as the centre of gravity passed in front of the line between the *points d'appui* of L_1 and R_3, which is the instant at which the insect falls to the right. When R_3 is placed on the ground again the area of support is bounded anteriorly by the line L_2, R_3, which passes just in front of the centre of gravity, because these two legs are protracted farther forwards than normally. Once again, these features suggest that mechanical factors are concerned in the modifications in posture and rhythm.

B. *The effect of cutting commissures*

The operations were carried out on anaesthetized cockroaches pinned ventral side uppermost. The thoracic ganglia are connected together by pairs of commissures which are visible through the thinner regions of the sternum. A small incision was made in this cuticle and a fine glass hook inserted beneath one of the commissures which was pulled out and severed with a fine pair of scissors. Body fluid was lost during the operation but the insects recovered within a few hours in the majority of cases. Altogether about thirty cockroaches were operated upon in this way. Films were taken at least 24 hr. after the operation.

In any experiment involving operations such as these it is often difficult to distinguish the effects produced by the specific operation from those resulting from the general injury. The results described below are the most common and are considered to represent the result of the interruption of central nervous pathways.

(1) *Single commissure cut*

These operations were nearly always successful and when observed the insects appeared to move normally. However, the films revealed changes in the rhythm.

For instance, in a male *Blatta* with the right pro-mesothoracic commissure cut, the legs of the uninjured side showed a perfect rhythm L_3, L_2, L_1 and this was true of the right side to some extent, but sometimes R_1 fell out of the rhythm. Even when both sides showed their normal rhythms (R_3, R_2, R_1, L_3, L_2, L_1), R_1 was again slightly out of time in that it protracted a little early and therefore moved before L_3, instead of after it as in the normal rhythm (Fig. 10B, ii).

In insects with a single meso-metathoracic commissure cut the hindleg of the operated side fell out of rhythm. In an insect with the right commissure cut the sequence L_3, L_2, L_1 was present throughout, but the legs of the right side, although observing the rule that no leg is moved before the one behind has taken up its supporting position, showed an altered rhythm as R_3 was very often protracted before R_1 (Fig. 10B, i). Other preparations have the hindleg very inactive unless the insect walks on sandpaper when it moves in a rhythm of its own and not very well co-ordinated with the other legs (Fig. 10B, iii).

(2) *Cutting both commissures*

This experiment is analogous to those carried out by Friedlander on earthworms which were cut in half, the two halves being connected by pieces of cotton. He found that the mechanical drag imparted to the posterior half by the anterior region was sufficient to enable the peristaltic wave to cross the gap. Similarly, activity in the two halves was co-ordinated when the nerve cord was the only connexion between the two. He concluded that both nervous and mechanical stimulation were important in bringing about the passage of the peristaltic wave.

In the cockroach the co-ordination of movement is much more seriously affected by the cutting of two as compared with the cutting of a single commissure. If both meso-metathoracic commissures are severed there is often a loss of depressor tone and the hindlegs do not touch the ground, presumably due to loss of connexion with the head. In favourable preparations, when the legs touch the ground, the hind pair of legs alternate in a slower rhythm than the other four legs, which continue in the normal rhythm. No definite co-ordination between the legs in front of the operation and those behind has been observed. Sometimes, however, when one of the hindlegs becomes excited ('spontaneously', by being touched with a brush, or by pressure on the abdomen) it pushes the body forwards. When this happens the three legs of that side show the rhythm—hind, middle, foreleg. This confirms the suggestion that when a leg takes up its supporting position the leg in front is protracted, because there will be a decrease in the proportion of the body weight which it supports. The conditions are similar to those operating when Pringle (1940) suddenly decreased the resistance to depression, the depressor muscles becoming less active.

These results are in agreement with those of Baldi (1924) and Ponz & Estartús (1951) who found no co-ordination between the hind pair of legs and the anterior ones when both commissures were severed. Ten Cate (1928, 1936), however, claimed that there was some co-ordination under these conditions. A possible explanation of these differences may be in the completeness of the separation effected between the two ganglia. There are, in addition to the two main commissures, two thinner ones which definitely contain large nerve fibres connecting the two ganglia, as has been observed in horizontal sections of the nerve cord. Hence, it is possible that only the two main commissures were cut in the experiments of ten Cate. Buddenbrock (1921) using *Dixippus*, and Roeder (1937) with *Mantis*, were unable to find any co-ordination when both commissures were severed, and concluded that mechanical drag was insufficient to bring about co-ordination in these insects.

Ten Cate (1941) carried out an interesting series of experiments involving the cutting of various combinations of commissures in cockroaches from which both middle legs had been removed. He found that the diagonal rhythm of movements persisted so long as there was a nervous path connecting the prothoracic and metathoracic ganglia. There appeared to be no specificity concerning this path as the same rhythm remained if both commissures on the right or left side were cut, or if the right pro-mesothoracic and left meso-methoracic commissures were severed. If both commissures between any two ganglia were cut there was little co-ordination between the two pairs of legs, even when the insect was held in contact with a rotating drum. Some of these striking observations have been confirmed.

These experiments upon four-legged insects emphasize the importance of non-specific pathways within the thoracic chain, and are in contrast to the results of the observations reported above on six-legged insects. In the latter experiments the manner in which the fore- or hindleg on the same side as the cut commissure protracted a little in advance, suggests that definite pathways have been cut which are normally of importance in regulating the timing of the leg movements. It must be remembered, however, that the change in the six-legged insect is a relatively slight one and that a small alteration in the timing of the leg movements of a four-legged cockroach would not be so noticeable because of the fewer legs. The two sets of observations are by no means inconsistent and, in fact, give an indication of the type of co-ordinating mechanism at work.

DISCUSSION

(*a*) *Plasticity of the nervous system*

The general significance of modifications in locomotory movements following the amputation of legs was emphasized by Bethe (1931) in his 'Plasticity theory of the central nervous system'. In this theory he drew attention to the ability of the central nervous system to produce new co-ordination patterns appropriate to the altered conditions. As the transpositions take place almost immediately, it is improbable that learning is involved and suggests that the neurological basis of the new patterns existed before the operation or were produced as a direct result of it. Bethe dismissed the first possibility because of the very large number of preformed patterns which appeared to be necessary to account for co-ordination following the removal of different combinations of legs. This is certainly true if each type of co-ordination has a completely different neurological basis, as the number would be $2^n - 2$, where n is the number of legs (Bethe 1930). Instead he suggested that the central nervous system was able to produce suitable co-ordination patterns within itself as a result of the operation. This was a radical change of outlook when compared with the then accepted chain reflex theories which regarded co-ordinated movements as a rigid sequence, each movement being produced reflexly by stimulation coming directly from the previously-acting leg. Although Bethe did not suggest a mechanism whereby such changes might take place, he pointed out that a mechanistic explanation in terms of 'a general regularity of the distribution of stimuli did not appear to be excluded'. Greater knowledge of proprioceptive mechanisms now makes such an explanation probable. It is not necessary to assume a different wiring diagram for each amputation since variations in activity of the

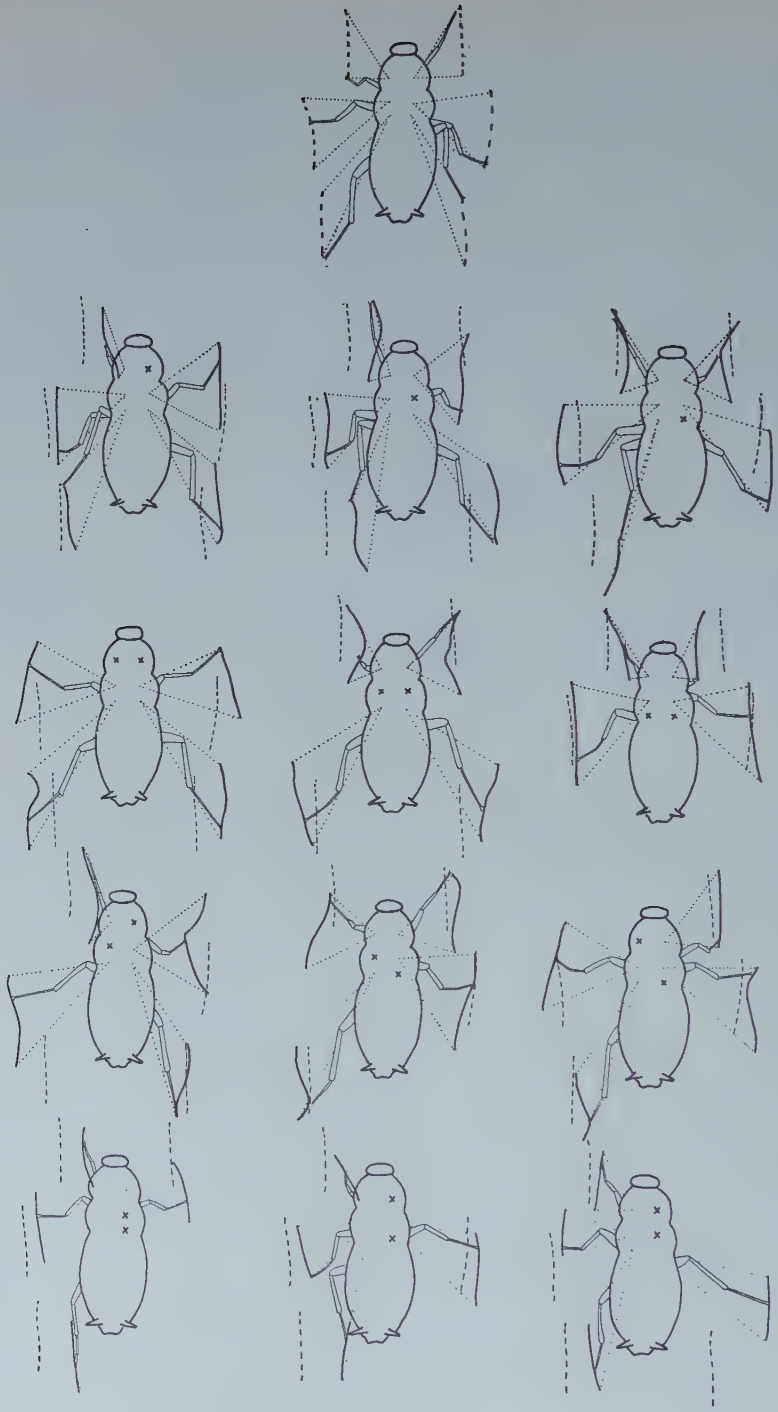

Fig. 11. Diagrams to show the changes in stride position which occur when different legs are amputated from a cockroach. Amputation of a leg is indicated by a cross on the thorax corresponding to its position. The positions occupied by the strides of the remaining legs in a normal insect are shown by interrupted lines, and their altered position following these operations is shown by continuous lines. Dotted lines indicate the angular movement of these legs which are drawn for one phase of the cycle.

remaining components is sufficient to account for the observed modifications. Thus, it would seem better to consider these plasticity changes as due to the operation of the same reflex mechanisms which produce co-ordinated movements in the normal insect. The altered mechanical conditions following the amputation of legs will change the sensory inflow, not only because of its absence from a given leg but also because of alterations in the excitation of sense organs in the remaining legs and other parts of the body, such as the intersegmental membranes (Diakonoff, 1936).

The results presented in this paper fit in well with such an interpretation since, in contrast to previous work, it has been shown that modifications take place in both posture and rhythm when only a single leg is removed. Examples have been given to show how some of these can be interpreted in terms of the known segmental reflexes. Furthermore, as each amputation produces a unique situation it is to be expected that the co-ordination and posture will likewise be unique, as has been found. Fig. 11 summarizes the results of the twelve different combinations in which one or two legs have been amputated from a cockroach. The altered path of the limb tips indicates how the posture of the legs is modified. In all cases there is a tendency for the remaining legs to take over the supporting function of the amputated legs. The legs adjacent to those amputated are usually the most affected, but changes in posture and rhythm occur to some extent in all of them. The total range in posture of a given leg is quite remarkable and must involve adjustments in the activity of many muscles. Similar changes in limb posture were described in the centipede *Scutigera* by Lissmann (1935).

Any interpretation of these modifications is bound to be incomplete until a great deal more is known of the mechanical conditions operating, and the precise mode of functioning of the different groups of campaniform sensilla described by Pringle (1938*b*). The reflex effects of stimulation of the hair plates (Pringle, 1938*c*) and of the widespread chordotonal sensilla which can have a proprioceptive function (Hughes, 1953) also remain to be discovered. However, in a general way it can be seen how the reflex mechanisms of the normal insect operating under changed conditions would produce the observed modifications in posture.

(*b*) *The rhythm of leg movements*

It is impossible to make any generalizations which will always enable one to forecast the rhythm adopted by the remaining legs when one or more legs are amputated, but certain features are usually recognizable. With extremely few exceptions it has been found that, as in the normal insect, no leg is lifted before the leg behind it on the same side is placed on the ground. The delay between these two events varies, however, and the phase relationship of the sequences on the two sides of the body are also variable. Thus two legs of a segment usually alternate but they may move almost simultaneously. Most of the rhythms can be produced by varying one or more parameters of the normal rhythm (e.g. $\frac{\text{protraction time}}{\text{retraction time}}$, phasing of two legs of a segment, etc.). For instance, the often-quoted transformation to the diagonal rhythm following the removal of both middle legs results if these legs are omitted from the normal rhythm in a slowly-moving cockroach, where p/r is about $\frac{1}{5}$.

Previous workers, without the use of ciné-analyses, have held that the diagonal order of lifting $\begin{array}{c|c} 1 & 3 \\ \hline 4 & 2 \end{array}$ is *always* found in a four-legged insect. In many cases this has been confirmed for the cockroach, but it is certainly not invariably true nor is the rhythm quite the same for any two amputations. Sometimes for instance, the four legs do not move at equal intervals of time after one another, one pair of diagonal legs often tending to move synchronously but not the other pair. Furthermore, the diagonal order of lifting (e.g. R_1, L_3, L_2, R_2, R_1) is not always the most common after a particular amputation and even when most prevalent it is often interrupted by other rhythms. In some cases, of course, the order of lifting in four-legged insects remains the same as in the intact insect, yet produces a diagonal rhythm (e.g. amputation of R_1 and L_1, or R_3 and L_3). Yet in some of these cases also, other rhythms are found which suggest that the normal function of the legs removed and the position of the centre of gravity with respect to the remaining legs are important factors which must be taken into consideration, and these will vary according to the amputation.

It is difficult, therefore, to agree with generalizations which suggest that the diagonal rhythm is invariably found in all instances when two legs are removed from an insect, but it is certainly the most common. The same is true of insects in which it is normal for only four legs to be used in walking. Thus a notable exception has been described by La Greca (1947) in the Acridiid, *Tropidopola cylindrica* which at slow speeds uses the rhythm L_2, R_2, $\begin{matrix} R_1 \\ L_1 \end{matrix}$, L_2, etc., or L_1, R_1, $\begin{matrix} R_2 \\ L_2 \end{matrix}$, L_1, etc., and at faster speeds limb pairs move synchronously in the rhythm $\begin{matrix} L_1 \\ R_1 \end{matrix}, \begin{matrix} L_2 \\ R_2 \end{matrix}, \begin{matrix} L_1 \\ R_1 \end{matrix}$. The tip of the abdomen provides an additional point of support during these movements which is not surprising as the centre of gravity must be quite far back in these insects. The importance of the tip of the abdomen for support was also pointed out by Wille (1924) in *Rhipipteryx chopardi* which uses only the front four legs in walking. In this insect the legs were lifted in a diagonal rhythm, i.e. R_1, L_2, L_1, R_2, R_1, etc., but usually only two legs were supporting at any one time and the triangle of support was completed by the tip of the abdomen.

The significance of the sequence in which the four legs are lifted in a tetrapod has been considered in detail by Gray (1944). He showed that, of the six possible sequences, the typical rhythm is the only one in which static stability is maintained throughout the cycle. This is only true when the four legs are distributed evenly about the centre of gravity. It is of interest, therefore, that many of the modifications in posture described above will tend to satisfy this condition. Furthermore, in those amputations where an even distribution cannot be achieved, it is to be expected that rhythms other than the diagonal pattern will be present. An example of this is shown by cockroaches without the two hindlegs, where rhythms similar to those described for *Tropidopola* have been observed. The relationship between rhythm, posture and the position of the centre of gravity with respect to the changing area of support (found in analyses of films), indicates that static stability tends to be maintained throughout the cycle as a result of modifications following the amputation of legs. That this is not always achieved is because it is sometimes physically impossible. It appears, then, that modifications of rhythm, as of posture, can be interpreted in terms of the altered mechanical conditions resulting from a given amputation.

Von Buddenbrock (1921) considered this explanation but rejected it in the light of an experiment with a four-legged *Dixippus*, in which the middle leg stumps were allowed to rest upon a small platform attached beneath the thorax. The rhythm of leg movements in such an insect was the same as in the normal insect, i.e. it did not show the diagonal sequence. It is important to notice that in this experiment the middle legs were amputated in the middle of the femur. The trochanter remained intact and inspection of this joint has confirmed the expectation that it would be provided with groups of campaniform sensilla and hair plates. Hence, the proprioceptive inflow from these legs could still take place, at least qualitatively, as in the normal insect. The timing of this inflow would be normal as the stumps tend to move in the normal sequence. This interpretation received support from experiments on *Dixippus* in which the middle legs were amputated proximally to the trochanter. Despite their small size it was possible to arrange for the coxae to move on the platform, and in all cases the remaining legs showed a diagonal rhythm. In his recent textbook von Buddenbrock (1953) describes a further experiment by Schaller (as yet unpublished) in which an insect holding a piece of paper with the two middle legs also showed the diagonal sequence. Here the normal timing of impulses from the middle legs would not be present, as is also true if the middle legs are rendered immobile by anaesthesia or by fixation of the joints. Under these conditions the insect moves as though the legs were removed, as was also shown in crabs by Kühl (1931).

Thus, it would seem that so long as there are only four legs in contact with the substratum the integration of their proprioceptive inflow tends to produce the diagonal rhythm, provided the legs are distributed evenly about the centre of gravity.

(*c*) *The co-ordination of movements*

In addition to the evidence discussed above, the following observations on normal insects also indicate the importance of mechanical factors in co-ordination.

(i) If a small weight is placed eccentrically on the metathorax of a cockroach it is found that the middle and particularly the hindleg on that side are displaced more laterally to the body. This effectively increases the lever arm of these legs so that equilibrium in roll will be maintained without any disproportionate increase in the vertical force operating at the tarsi of these legs. These changes in posture are similar to those which occur when a single middle leg is removed from the insect.

(ii) The paths of the limb tips relative to the head in a normal insect are parallel to the axis of progression, and this also indicates a mechanism balancing the distribution of forces exerted by the legs on the ground. Some preliminary measurements of the vertical forces operating at the tarsi during normal movement confirm this conclusion.

(iii) When studying the geotactic responses of *Tetraopes* and other insects, Crozier & Stier (1929) found that the angle at which they walked up an inclined plane was precisely related to its angle of inclination. They suggested that this was brought by some mechanism whereby 'the tension excitations due to the pull of the animal's weight are the same, within a threshold difference on the two sides of the body'.

These observations suggest that during movement the forces at the tarsi tend to be equalized on the two sides of the body. The campaniform sensilla are probably of great importance in this mechanism since their distribution will enable them to detect such forces (Pringle, 1938*b*). The hypothesis is suggested that the altered postures and movements described in this paper tend to produce an equal inflow of impulses from these and other proprioceptors on the two sides of the body. It is reasonable to suppose that the central nervous system has a mechanism for comparing the inflow from the two sides, and in these cases produces compensating movements which tend to equalize the feedback for these sense organs.

From the considerations presented in this paper it is suggested that the following factors are of importance in determining the co-ordination of insect movements.

(1) The mechanical drag imparted by the other legs. The importance of this factor was emphasized by ten Cate (1928, 1936), but that it is insufficient by itself is indicated by the absence of co-ordination when both meso-metathoracic commisures were severed.

(2) The proportion of the body weight which a leg supports. This may be decreased by the leg behind taking up a supporting position or increased by the protraction of adjacent legs. The presence of the sequences L_3, L_2, L_1 and R_3, R_2, R_1 will be maintained by this factor.

(3) It is suggested that there is an overall co-ordination between thoracic ganglia regulating movements of all legs, such that feedbacks indicating components of forces acting at the tarsi will be balanced on the two sides of the body.

(4) Segmental reflexes objectively demonstrated by Pringle (1940) are of basic importance in all these reactions.

(5) Intersegmental effects, whose exact nature is undefined, are indicated by several experiments such as when a single commissure is cut in the intact insect and distinct pathways appear to be disrupted. Intersegmental reflexes have not been demonstrated objectively, although some indication of their presence was found in experiments involving stimulation of cockroach ganglia with direct currents (Hughes, 1952*b*). Some recent observations by D. M. Maynard (personal communication) have also indicated the presence of such pathways. Thus, electrical stimulation of sensory nerve fibres entering the mesothoracic ganglion produces a response in the muscles of the foreleg on the same side more regularly than in the contralateral foreleg. Such pathways would facilitate the sequence hind-, middle, fore- of ipsilateral legs.

(6) The supra- and sub-oesophageal ganglia have an overall effect upon the thoracic ganglia since co-ordinated movements in the strict sense are not present when they are extirpated (Baldi, 1924).

In the light of this discussion insect movement becomes an extremely complex interaction of reflexes most of which reinforce one another. This stress on the role of peripheral sense organs has been justified in the locomotion of both amphibia and fish (Gray 1950, Lissman, 1946*a*, *b*), but other workers (see S.E.B. Symposium, 1950) have emphasized the importance of intrinsic factors within the central nervous system in determing the patterns of movement independently of the peripheral inflow. Bethe's original theory attributed considerable executive properties to the central nervous system, though few people would now accept the suggestion that it was able to 'invent' new patterns so quickly. Weiss (1950) has criticized this aspect of Bethe's theory, but is prepared to accept the presence of pre-existing mechanisms capable of producing the new co-ordination patterns. He accepts plasticity on the 'intermember' but not on the 'intramember' level, but we have seen that there are adaptive changes in the degree of activity of different muscles within a limb as well as changes in the rhythm of leg movements. However, we must obviously accept the existence of certain preformed tracts and synaptic connexions within the central nervous system, but this does not necessarily mean that these pathways determine the pattern of movements; rather, they are the means by which patterns of peripheral stimulation entering from different sense organs become integrated with one another. The relevance of such observations to current concepts of instinctive behaviour has been considered by Thorpe (1948), and the results described in this paper together with unpublished observations on the swimming of *Dytiscus*, support his conclusions in that they throw some doubt on the physiological basis of these concepts as applied to insect locomotion.

SUMMARY

1. The effects of limb amputation and the cutting of commissures on the movements of the cockroach *Blatta orientalis* have been investigated with the aid of cinematography. Detailed analyses of changes in posture and rhythm of leg movements are given.

2. It is shown that quite marked changes occur following the amputation of a single leg or the cutting of a single commissure between the thoracic ganglia.

3. Changes following the amputation of a single leg are immediate and are such that the support normally provided by the missing leg is taken over by the two remaining legs on that side. Compensatory movements are also found in the contralateral legs.

4. When two legs of opposite sides are amputated it has been confirmed that the diagonal sequence tends to be adopted, but this is not invariably true. Besides alterations in the rhythm which this may involve, there are again adaptive modifications in the movements of the limbs with respect to the body.

5. When both commissures between the meso- and metathoracic ganglia are

cut, the hind pair of legs fall out of rhythm with the other four legs. The observations on the effects of cutting commissures stress the importance of intersegmental pathways in co-ordination.

6. It is shown that all modifications following the amputation of legs may be related to the altered mechanical conditions. Some of the important factors involved in normal co-ordination are discussed, and it is suggested that the altered movements would be produced by the operation of these factors under the new conditions. It is concluded that the sensory inflow to the central nervous system is of major importance in the co-ordination of normal movement.

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STUDIES ON THE FEEDING AND NUTRITION OF *TUBEROLACHNUS SALIGNUS* (GMELIN) (HOMOPTERA, APHIDIDAE)

I. THE UPTAKE OF PHLOEM SAP

By T. E. MITTLER
*Department of Zoology, University of Cambridge**

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It has generally been supposed that the uptake of large quantities of plant sap by aphids is the direct result of suction. Zweigelt (1914) has questioned this concept, and Kennedy & Mittler (1953) have thrown further doubt on the supposition that aphids are typical 'sucking' insects. The present paper sets out results, obtained in a study of the uptake of phloem sap by *Tuberolachnus salignus* (Gmelin), which may help to clarify this question.

MATERIALS AND METHODS

The experimental aphid, *T. salignus*, is only known to occur in the apterous and alate parthenogenetic viviparous forms. The species was reared throughout the year on stem-cuttings of *Salix alba* L. 2–3 ft. long. These were planted in sandy soil in 7 in. clay pots and were kept in a greenhouse at a mean temperature of 20° C. under a photoperiod of 16 hr. Non-dormant stems readily rooted and produced leafy shoots within 1 or 2 weeks. Dormant stems were stimulated into growth by treating them with a rooting hormone (Seradix 3) and by applying some bottom heat to the pots. The culture was started by placing some naturally infested willow branches over rooted stem-cuttings in three pots. After a week three further pots were set up in close proximity to these, and new aphid colonies rapidly established themselves. A weekly production of several hundred apterous adult aphids was maintained by adding three pots of willow stems to the culture each week and discarding these after every 4 or 5 weeks. A working culture consisted of 12–15 pots. As needed, a hundred or more apterous adults 1–2 days old were obtained from the stems and from the rim of the pots where they tended to wander. The aphids were collected individually by means of a camel-hair brush or by the use of forceps with flattened tips.

Several species of *Salix* were used as experimental host plants for *T. salignus*. Unless otherwise stated 2–4-year-old trees of *S. acutifolia* Willd. (also known as *Salix daphnoides* Vill.), with straight main stems 5–6 ft. long, were used throughout these studies. This species was found to support the heaviest naturally occurring infestations of *T. salignus*, and was readily obtainable for potting.

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* Present address: Science Service Laboratory, Department of Agriculture, St Jean, P. Quebec, Canada.

To determine the course taken by the stylet bundles of *T. salignus* in the stems of *S. fragilis* L. the proboscides of feeding aphids were severed according to the method outlined by Kennedy & Mittler (1953). Pieces of the stem containing the stylet bundles were fixed in Carnoy's fluid, cut into transverse and longitudinal tangential sections, and stained according to the method of Rawitscher (1933).

The relative positions of the tips of the mandibular and maxillary stylets of *T. salignus* at different stages of their insertion into willow stems were determined by withdrawing, by means of forceps, severed embedded stylet bundles and examining these microscopically.

To assess the contribution made by the pressure of the phloem sap in willow stems to the rate of sap uptake by normally feeding *T. salignus*, the rate at which aphids excrete honeydew and the rate at which sap exudes from the cut ends of their embedded stylet bundles were simultaneously estimated. This was achieved by collecting and measuring the volume of honeydew excreted in an hour by a colony of aphids of the same age, and by collecting and measuring the volume of sap exuded in 5–8 hr. from the severed stylets of one or more of the aphids in the colony. Measurements of the radius of the food canal within the stylets of adult *T. salignus* were obtained from electron-microscope photographs taken of the stylets. The length of the stylets was measured under a binocular microscope. The viscosity of phloem sap passing through the stylet food canal was estimated by measuring the total sugar concentration of the sap by the method of Morris (1948). An estimate of the osmotic pressure of the phloem sap was also obtained from a determination of the total sugar concentration.

In order to determine whether the ingestion of sap by *T. salignus* is an active process, colonies of feeding aphids were rapidly anaesthetized so that the aphids had no time to withdraw their inserted stylets. This was achieved by directing a gentle stream of carbon dioxide gas on to the colonies. Aphids were maintained anaesthetized by this means for 24 hr.

To test the effect of wilting and loss of turgor of the host plant on aphid feeding, the foliage of some potted willows was induced to wilt by allowing the soil to dry out over a period of several days. Wilting and loss of turgor were also induced by making deep incisions into the bark of *S. alba* stems about small colonies of *T. salignus*, and gently prising the bark away at its cambial layer. This procedure did not disturb the feeding aphids directly.

The dormancy of potted *S. acutifolia* trees, which in winter were kept outdoors, was broken within 2–3 weeks by bringing the plants into the greenhouse.

RESULTS

(*a*) *The disposition of the stylets in the host plant*

The stylet bundles of feeding *T. salignus* were found to pass inter- and intracellularly through the cortical tissues of *S. fragilis* stems and to end in the newly differentiated phloem sieve-tubes adjacent to the cambium. It may be noted that investigations by Horsfall (1923), Davidson (1923), Smith (1926), Rawitscher (1933), Romell (1935), Grossmann (1937), Tate (1937), Roberts (1940), Leonhardt (1940) and Lindemann (1948) have shown that a large number of other aphid species also insert their stylet tips into the phloem sieve-tubes of their host plant.

The stainable sheath of saliva, which surrounds the stylet bundles within a willow stem, was found to extend beyond the surface of the willow bark in the form of a small cone about the stylet bundles. Within cells the sheath had a smooth outline, but within intercellular spaces it had a beaded appearance. A small accumulation of stainable saliva was occasionally found where fibre bundles diverted stylets from their radial course to the phloem. Salivary sheaths in the phloem were occasionally observed to branch. An accumulation of stainable saliva was never detected beyond the innermost limit of a stylet bundle in the phloem.

Microscopic examination of the stylet bundles of feeding aphids showed the curved tips of the maxillary stylets to be apposed to one another, and the serrated tips of the mandibular stylets to be staggered and a short way behind the former. The observation by Leonhardt (1940), that the food and salivary canals do not terminate at the extreme tips of the maxillary stylets, was confirmed. The canals appeared to open into a lenticular cleft a short way behind their apposed tips. Stylet bundles in various stages of insertion showed the tips of the maxillary and mandibular stylets to be staggered.

The extent to which the stylets of feeding *T. salignus* are inserted into the stems of several species of *Salix* was found to be related to the age of the stems. Whereas only the distal end of a stylet bundle is inserted by an aphid feeding on 1-year-old stems, the entire length is inserted into 5–6-year-old stems. In 2–4-year-old stems stylet bundles are inserted for one-third to one-half of their length. It may be noted that the distance between the surface of a willow stem and the newly differentiated phloem elements adjacent to the cambium increases each year due to the secondary thickening of the stems.

(*b*) *The mechanism of sap uptake*

The radius of the stylet food canal of adult *T. salignus* was found to be $1\cdot8\mu$ near the aphid's head and $0\cdot6\mu$ at the distal end. The stylets of adult *T. salignus* were found to be 1·8 mm. long, and the viscosity of the phloem sap passing through the stylet food canal was estimated to be close to 1 centipoise. Measurements of the rate of sap exudation from severed stylets and of the rate of honeydew excretion from feeding aphids showed the normal rate of flow of sap through the stylet food canal of adult aphids to lie between 1 and 2 cu. mm. per hour. Applying Poiseuille's relationship to the flow of phloem sap through the stylet food canal of aphids, it was calculated that the pressure required to maintain a rate of flow of 1–2 cu. mm. per hour through the stylet food canal of a feeding adult *T. salignus* is 20–40 atm.

Measurements of the total sugar concentration of exudates from severed stylets indicate that the osmotic pressure of the phloem sap of *S. acutifolia* is at least

5 atm. and probably considerably greater than 10 atm. One may note that Münch (1930) and Dixon (1933) have shown the phloem sap of *Quercus*, *Robina* and *Fraxinus* spp. to have an osmotic pressure of 20·9 to 37·3 atm.

T. salignus, which were feeding in colonies on the main stem of *S. acutifolia* trees, dispersed and made repeated efforts to reinsert their stylets into the stems as soon as the foliage of the trees showed signs of wilting. The leaf-feeding aphid *Cavariella aegopodii* (Scop.), which was also present on these trees, dispersed at the same time.

T. salignus inserted their stylets for short periods into dormant willow stems, but did not excrete honeydew and presumably did not feed. As soon as the dormancy of the plants was broken and bud swelling occurred the aphids began to feed normally on the stems.

T. salignus ceased to excrete honeydew but maintained their stylets inserted for 2–3 hr. in pieces of bark after these had been stripped from the cambial layer of *S. alba* stems.

In a like manner, *T. salignus* feeding on a *S. fragilis* tree ceased to excrete 2–3 hr. after stems on which they were feeding were cut into 5–6 in. lengths. The aphids, however, maintained their stylets inserted for 5–6 hr. Exudation of sap from severed stylets also ceased 2–3 hr. after the stems were cut. This is in contrast to the unabated exudation of sap for more than 24 hr. frequently observed from severed stylets embedded in intact stems of various *Salix* spp. A large droplet of sap could be made to exude from severed stylets which had ceased to exude by pressing on the bark within 1 cm. of the stylets. On releasing the pressure the droplet was withdrawn into the plant through the stylets, showing that cessation of sap exudation was not due to blockage of the stylets but due to insufficient pressure within the plant.

Table 1 sets out the results of experiments in which the average rate of honeydew excretion of *T. salignus* in each instar, and the average rate of sap exudation from their severed stylets were simultaneously determined.

Table 1. *Average rate of excretion of honeydew by* Tuberolachnus salignus *in each instar and the average rate of sap exudation from their severed stylets at* 20° C.

Instar	Average rate of excretion (cu. mm./hr.)	Average rate of sap exudation (cu. mm./hr.)
First	0·45	0·64
Second	0·73	0·72
Third	0·94	1·05
Fourth	1·36	1·46
	1·43	1·47
Adult apterous	2·06	1·89
	1·87	1·75
	1·82	1·80
	2·08	1·82
	1·84	1·89
	1·71	1·79

Feeding aphids which were subjected to carbon dioxide anaesthesia for 24 hr. did not exude sap from any part of their body nor did they become distended. When their stylets were severed after this period sap exuded readily from the embedded stylet stumps showing that the pressure was maintained within the stylet food canal.

CONCLUSIONS AND DISCUSSION

The fact that *T. salignus* and other aphid species insert their stylet tips into the phloem sieve-tubes of their host plant suggests that the insects ingest sieve-tube sap in preference to other plant sap. A high nutrient value of the sieve-tube sap has generally been supposed to be the basis of the aphid's choice. The pH value of the sieve-tube sap has also been held responsible for its preferential selection by other plant-feeding Hemiptera (Fife & Frampton, 1936).

Although aphids may choose the sieve-tube sap as food because of its taste or nutrient value their choice may have a different basis. When an aphid inserts its stylets into a plant numerous cells are pierced in succession. The vacuolar sap of each of the cells is under a certain turgor pressure, and as the cells are pierced some of their sap is forced into the stylet food canal. As the cortical cells contain only a limited amount of sap under pressure they can only force a very small volume of nutrient sap up the stylet food canal of a feeding aphid. The phloem sieve-tubes, on the other hand, whose contents are in some form of organic continuity (Crafts, 1939; Esau, 1939), may be compared to an extensive system of anastomosing 'pipe-lines' which may supply an aphid with large amounts of nutrient sap under undiminishing pressure. The efficiency with which such a system supplies an aphid with the sap it ingests can be gauged from the fact that an uptake of 1 cu. mm. of sap per hour by one aphid from a single phloem sieve-tube cell indicates that a volume of sap 100,000 times greater than the capacity of the cell passes through it in 1 hr. This may involve a rate of sap flow of 500 cm. per hour within the phloem sieve-tubes of a willow stem which is comparable to the highest rates of translocation listed for other plants by Tammes (1952).

It therefore appears that aphids use the phloem as a feeding site mainly because this tissue has the greatest capacity for *maintaining* a supply of nutrient sap under pressure.

It may be suggested that the stainable saliva which forms the stylet sheath is only injected into a pierced cell after the turgor pressure of its sap has been reduced by the entry of some of the sap into the stylet food canal. When stylets pierce a phloem sieve-tube and the pressure is maintained aphids do not pump (or are unable to pump) stainable saliva down the salivary canal of their stylets, although the stylet tips may remain in a sieve-tube for many hours. It seems probable that phloem sap enters the stylet food canal of a feeding aphid through the cleft between the curved tips of the maxillary stylets when they are apposed to one another.

The occurrence of branched salivary sheaths in the phloem of a plant has been interpreted by Zweigelt (1914) to indicate that an aphid may simultaneously obtain sap, in a haustorial-like manner, from all the branches its stylets have made in the phloem. This phenomenon may, however, also be interpreted as an aphid's response to a local exhaustion of phloem sieve-tube sap, or lack of sufficient pressure for the aphid to be able to maintain its normal feeding rate. It appears to be a convenient method for an aphid to seek out and tap different parts of the phloem of its host plant successively, without it having to withdraw its stylets completely and to reinsert them elsewhere.

The observations that *T. salignus* do not feed on dormant willows and that they seek out the newly differentiated phloem sieve-tubes adjacent to the cambium of secondarily thickened willow stems, further support the view expressed by Kennedy, Ibbotson & Booth (1950) that aphids require to tap a fully functional 'pipe-line' in order to obtain sufficient sap.

As it is questionable whether an aphid could produce a suction pressure of even 1 atm., and as calculations have shown that a pressure of 20–40 atm. may be necessary to maintain the normal rate of flow of sap through the stylet food canal, one may suppose that an aphid would not be able to feed at its normal high rate by its own sucking efforts.

Although aphids may be able to suck up a limited amount of fluid when it is not under pressure, as artificial feeding experiments have indicated (Hamilton, 1935; Maltais, 1952), the results suggest that aphids depend on the natural turgor pressure of their host plant for their normal feeding. This pressure is responsible for causing the exudation of sap from severed embedded stylets and may be assumed to be operative in forcing sap up the stylet food canal into intact feeding aphids. It appears that the rate at which the host plant forces the sap up the stylet food canal determines the rate of sap uptake and excretion of the aphids. In order to ingest the sap aphids must, however, actively swallow the sap forced up their stylet food canal even though they do not have to exert, or are unable to exert, very much suction on it.

One may speculate that the differences in the rates of exudation from the severed embedded stylets of the various instars are due to differences in the dimensions of their stylet food canal.

Although a low concentration of certain organic constituents in phloem sap may necessitate the ingestion of as much sap as possible, the excretion of large quantities of amino-acids (Maltais & Auclair, 1952; Mittler, 1953) suggests that the host plant is forcing more nutrient sap into feeding aphids than the insects are able to absorb.

A theory of aphid feeding based on a supposed physico-chemical action (*exosmosis*) of aphid saliva on the cells of a plant, thereby making sap available under pressure to aphids, has been proposed by Zweigelt (1914). Although very little evidence exists to support this theory it is interesting to quote some of Zweigelt's conclusions: 'The complicated action attributed to the muscles of the pharyngeal suction pump overrates the efforts of the insect, which merely has to swallow and not to suck.' The present investigation supports these conclusions but rejects the concepts on which they are based. The generally accepted view that an aphid's normal mode of feeding is by suction is, however, being challenged.

SUMMARY

1. A study has been made of the factors involved in the uptake of phloem sap by *Tuberolachnus salignus* (Gmelin) feeding on the stems of various *Salix* spp.

2. A method has been developed for maintaining the parthenogenetic viviparous forms of *T. salignus* in culture throughout the year.

3. It has been established that during normal feeding *T. salignus* have the tips of their stylets inserted into the phloem sieve-tubes of the host plant.

4. The phloem sieve-tube sap of intact and turgid willow stems is under considerable pressure. This pressure forces the sieve-tube sap up the stylet food canal of feeding aphids, and also causes the sieve-tube sap to exude for many hours from the cut end of embedded stylet bundles.

5. Intact and feeding *T. salignus* rely almost entirely on this pressure to maintain their normal rate of sieve-tube sap uptake. The aphids must, however, swallow actively in order to ingest.

The work reported in this series of papers on the feeding and nutrition of *Tuberolachnus salignus* was carried out during 1950–3, and forms part of a thesis for the Ph.D. degree of the University of Cambridge. I am indebted to Prof. V. B. Wigglesworth and Dr J. S. Kennedy, for their counsel and encouragement. I am grateful to many members of the University of Cambridge for advice and facilities, and to the Agricultural Research Council for financial support. Dr E. J. LeRoux kindly read and criticized the manuscripts.

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POLARIZED LIGHT AND THE ORIENTATION OF TWO MARINE CRUSTACEA*

By RICHARD BAINBRIDGE
Zoological Laboratory, University of Cambridge
AND TALBOT H. WATERMAN
Department of Zoology, Yale University

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INTRODUCTION

Naturally occurring polarized light is used for orientation by many arthropods. Various insects and chelicerates as well as crustaceans, employing either their simple or compound eyes, can detect the pattern of linearly polarized light in the blue sky and from it determine geographical direction. Among Crustacea, the semi-terrestrial beach amphipod *Talitrus* and the isopod *Tylos* show this when, on being disturbed, they are able to head directly towards the sea without using other clues (Pardi & Papi, 1953*a*, *b*; Pardi, 1954). Like that of the sky, underwater scattered illumination is known to be polarized in a way primarily determined by the sun's position (Waterman, 1954*a*, 1955; Ivanoff, 1956; Waterman & Westell, 1956).

The possibility thus exists that *aquatic* arthropods, or other underwater forms sensitive to polarization, may use this submarine light pattern as a similar sort of sun compass (Waterman, 1957). It is certain that some aquatic arthropods can in fact perceive polarized light (Verkhovskaya, 1940; Waterman 1950; Baylor & Smith, 1953; Eckert, 1953), but few quantitative data are available. No direct evidence linking this perceptive ability with their normal orientation and migration has yet been found, although an effect of sky polarization observed underwater has been suggested as a possible factor in the diurnal vertical migration of plankton (Verkhovskaya, 1940). This paper describes an experimental approach to the general problem. The major part of the work comprises a quantitative laboratory study of the responses of a marine mysid to plane polarized light, and this is supplemented by an exploratory field project in which a caridean decapod was found capable of swimming straight courses in open water where landmarks were not available.

LABORATORY EXPERIMENTS ON *MYSIDIUM*

Organisms

In seeking suitable experimental animals with which to study quantitative effects of polarized light on orientation and behaviour many species have been tested. Exploratory, and as yet equivocal, tests have been made on a number of teleosts, both marine and fresh water, but mainly marine crustaceans have been used.

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* Contribution No. 231 from the Bermuda Biological Station.

Littoral Mysidacea, Natantia and Reptantia of many varieties, Stomatopoda, pelagic and bathypelagic Copepoda, Amphipoda and Caridea have all been examined for favourable reaction patterns. So far zoeas of littoral Brachyura and the adult mysid, *Mysidium gracile* (Dana) have offered the most promise. This report is concerned mainly with the swimming behaviour of the latter form in vertical beams of linearly polarized light. Distinctive features which make this animal appropriate for such work are its sustained locomotor activity and its strongly visual reaction patterns. The latter are mediated through the large and well-developed stalked compound eyes, a median eye being absent in mysids (Mayrat, 1956) as apparently throughout the Crustacea Peracarida. Adult specimens about 5–10 mm. in length, either freshly caught or kept in sea-water aquaria, were used in the experiments. No effect of length of captivity was found in the mysid response patterns; animals were used for experiments after periods of captivity ranging from 1–2 hr. up to about 2 weeks.

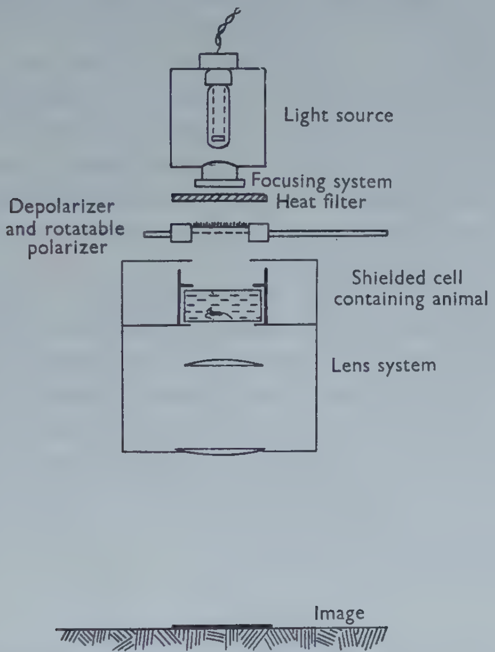
Light source
Focusing system
Heat filter
Depolarizer and rotatable polarizer
Shielded cell containing animal
Lens system
Image

Fig. 1. Diagram of apparatus used to expose *Mysidium* to a vertical beam of nearly 100% linearly polarized light and to record the resultant swimming orientation.

Apparatus

A small experimental cell containing the animal in sea water was illuminated from above by a vertical beam of polarized light whose plane of polarization could be altered at will. This provided an illumination of 700 lux at the cell. Originating from a ribbon filament projection bulb the light passed successively through a focusing lens system, a heat filter, a depolarizer, a rotatable Polaroid linear polarizer, the experimental cell, and finally two lenses which produced an image of the latter and its contents upon the bench beneath (Fig. 1). The apparatus was set up in a darkroom with a suitable arrangement of baffles and shields to prevent extraneous light from reaching the experimental cell. This consisted of a short length of cylindrical plastic tube 50 mm. in diameter sealed to a flat glass plate. The latter ensured a perfectly plane bottom which was important optically for good image projection and also for avoiding the possible influence of an uneven surface on the animal's behaviour. The circular shape was also critical. Preliminary tests in a square cell showed a marked and statistically significant tendency of the mysids to swim at an angle of 45° to the sides whether the light was polarized or not. The effects of the container thus masked any effect due to polarized light, and such a cell was inappropriate for experiments of this nature.

To minimize polarization artifacts several important precautions were taken. The interposition of a depolarizer, made of two sheets of waxed paper, has already been mentioned. This cut out any intensity modulation arising from the slight inherent polarization in the light source. In addition, surface reflexions from the walls of the cell were greatly reduced. These would otherwise act as an analyser of polarized light striking them obliquely. To avoid this the vertical walls of the experimental cell were given an even, non-reflecting opaque surface by coating them with a thin layer of carbon black suspended in molten wax. In addition, the possibility of reflexion was virtually eliminated by shading the walls from direct light with a circular diaphragm having an aperture 5 mm. less than the inner diameter of the cell. Control experiments described below (p. 353) tested the need for and the effectiveness of this diaphragm.

Finally, the meniscus at the margin of the water surface was eliminated in order to remove the regular pattern of light and dark spots formed by the polarized light striking this boundary and readily visible at least to the human eye. To achieve this the upper edge of the cell walls was ground smooth so that a flat glass plate acting as a cover would seal off the completely full vessel without an air-water interface. Inspection of the whole optical set-up for spurious polarization effects was made with the special interference analyser described by Waterman (1955). This is sensitive down to about 5% polarization and showed that the apparatus as used was essentially free from polarization artifacts.

Procedure

In a typical experiment the cell was filled with fresh clean sea water, a single animal was introduced and the cover put on, care being taken to exclude air bubbles. The cell was placed symmetrically in the light beam and surrounded by a cylindrical black shield. In this position it was enclosed on all sides by the black-walled observation chamber about 25 cm. square. A clear image of the animal was focused upon a sheet of squared paper placed on the bench beneath. The lens setting, depending upon the depth of the mysid in the water, could be altered as the experiment proceeded. After an interval to allow the animal to settle down, the polarizer was rotated by the operator to one of seven positions. The planes of polarization (defined by the **e** vector) customarily used were 30° apart, starting clockwise from the horizontal direction of the recording grid as 0°. The particular plane used for a given determination was selected with the aid of a table of random numbers. It was unknown to the observer until a whole session of measurements was over.

To begin, the observer drew a line along the antero-posterior axis of the image of the mysid. Then at 5 sec. intervals this recording of its position was repeated for a total of 25 times. After that the polarizer was set at another randomly chosen angle of the seven and a further run of twenty-five positions was recorded. At the end of five such series of observations with different planes of polarization, operator and observer were interchanged and a second series of five similarly selected settings of the polarizer were tested. In the following discussion each group of twenty-five observations recorded for one plane of polarization is termed a 'run' and each group of ten runs is termed a 'set', which thus comprises 250 orientation measurements.

During such a recording period the animal would generally be swimming about in the cell fairly quickly. Some dexterity and experience were often required to fix its orientation exactly at each reading, but generally this was easy if the operator announced the time for recording and the observer had only to keep his eyes on the moving image. Every effort was made by the observer to keep his mind as blank as possible in order to avoid the chance of bias. Any possible influence of the first few lines drawn on the orientation chosen for later ones, or of guesses of the polarization plane were steadfastly resisted.

Analytical method

To detect any influence of the plane of the **e** vector upon the course set by the animal, the orientation of each line in a run of 25 was first measured with reference to the horizontal grid lines on the paper. The deviation of each such line (representing the animal's longitudinal axis) from the plane of polarization was then calculated. The frequency distribution of the resulting angles between 0° and 90° indicates any relationship between the animal's course and the plane of polarization. If there is no such relationship, a random distribution would be expected. But provided that orientation is generally in the plane of polarization, then a majority of readings in the neighbourhood of 0° might be expected; if at right angles to the plane of polarization, then in the neighbourhood of 90°. Most of the results are depicted in histograms representing 15° groups of angles between 0° and 90°. The data generally are insufficient to permit a more detailed analysis with smaller groups of angles. However, the large numbers of measurements in Figs. 3 and 4 are plotted for intervals of 5°.

Results

To illustrate these experiments in detail the results of two typical runs selected from a series with one animal are presented first (Fig. 2). Facsimiles of the laboratory records obtained with polarization planes 60° (A 1) and 150° (B 1) to the reference direction show the axial orientation of mysid at the end of twenty-five 5 sec. intervals each. The angles between these axial lines and horizontal (0°) in the reference grid are listed in Table 1, along with their differences from the plane of polarization. For analysis these deviations have been grouped into intervals of 15° (A2, B2) and 30° (A3, B3).

A clear bias towards an orientation at right angles to the plane of the **e** vector is apparent in these histograms. If the appropriate χ^2 test is applied to the numbers of readings falling into the various angular groups, the reliability of this inference may be tested statistically (for 30° intervals):

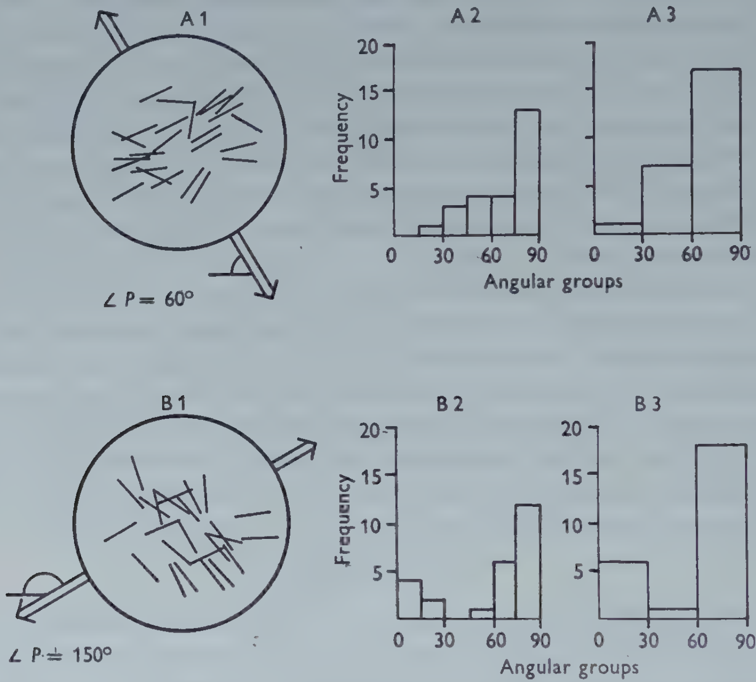

Fig. 2. Two sample runs (twenty-five observations each) of the swimming orientation of one *Mysidium* in a vertical beam of plane polarized light. In series A the **e** vector was at 60° relative to the horizontal reference direction (0°); in series B it was at 150°. A1 and B1 are facsimiles of the laboratory record made of the runs. Each of the twenty-five lines in the circle represents the position of the animal's longitudinal axis at intervals of 5 sec. Their deviations from the **e** vector planes are plotted in the histograms grouping angles in 150° intervals (A2, B2) and 30° intervals (A3, B3). χ^2 tests show the preponderance of orientation near 90° to be significant in both cases.

e vector	χ^2	Degrees of freedom	P
60° polarization plane	15·4	2	$<0\cdot01$
150° polarization plane	18·2	2	$<0\cdot01$

These values of P are within the limits normally considered significant. For the 15° intervals the values are within the significant range by an even wider margin. The data for the 150° polarization plane, in addition to the predominant 90° orientation, show some preference for swimming parallel to the **e** vector when not at right angles to it.

Table 1. *Angular measurements relating to Fig. 2*

Series A			Series B		
Serial no. of observation	Axial orientation of *Mysidium* relative to 0°	Angular deviations from plane of polarization	Serial no. of observation	Axial orientation of *Mysidium* relative to 0°	Angular deviations from plane of polarization
1	153	87	1	65	85
2	180	60	2	63	87
3	125	65	3	55	85
4	100	40	4	54	84
5	149	89	5	72	78
6	34	26	6	51	81
7	178	62	7	44	74
8	160	80	8	154	4
9	139	79	9	45	75
10	160	80	10	89	61
11	172	68	11	42	72
12	155	85	12	55	85
13	153	87	13	158	8
14	11	49	14	18	48
15	29	31	15	49	79
16	146	86	16	68	82
17	139	79	17	37	67
18	29	31	18	49	79
19	173	67	19	153	3
20	13	47	20	177	27
21	148	88	21	175	25
22	151	89	22	35	65
23	142	82	23	147	3
24	157	83	24	74	76
25	120	60	25	48	78

Table 2. *Summary of the distribution of angular deviations of the axis of* Mysidium *from the plane of polarization*

	Percentages in angular groups						No. of observations
	0–15°	16–30°	31–45°	46–60°	61–75°	76–90°	
Total results (24·5 sets)	12·6	12·6	14·7	18·2	20·3	21·6	6125
	25·2		32·9		41·9		
9 Non-significant sets	15·6	16·4	17·9	17·4	16·2	16·5	2250
	32·0		35·3		32·7		
15·5 significant sets (see Fig. 3)	11·1	10·2	12·7	18·9	22·5	24·6	3875
	21·3		31·6		47·1		
22 significant runs (29 Aug.) (see Fig. 4)	8·7	6·4	8·2	14·9	26·7	35·1	550
	15·1		23·1		61·8		

N.B. The χ^2 for the numbers in the non-significant sets is 5·4 making *P circa* 0·4 which is not significant. All the other χ^2's are highly significant.

The total data presented in this report comprise 245 runs of the kind illustrated above or 6125 orientation measurements made in polarized light plus a number of controls in unpolarized light. In the sum total of all these results, as in the sample already presented, *Mysidium* shows a statistically significant preponderance of swimming perpendicular to the **e** vector (Table 2). Some further interesting relations become obvious if the data are considered in more detail. For instance, of the 24·5 sets which might have been expected to give 90° polarized light orientation, 15·5 did so, while the remainder showed a random orientation; thus demonstrating that under these conditions, the animal will react to the polarization pattern only about 60% of the time.

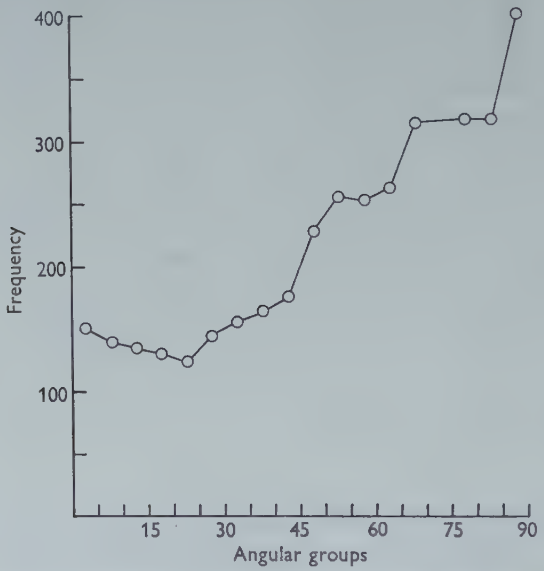

Fig. 3. Over-all swimming orientation of *Mysidium* (15·5 significant sets, 3875 measurements) relative to the **e** vector plane of the vertical beam of polarized light. Clear preponderance of alignment perpendicular to the **e** vector is shown with a minor rise parallel to the plane. The accuracy of the polarized light orientation appears to be low and the incidence of random orientation rather high.

A more precise idea of the response when it does occur can be obtained if the 15·5 significant sets are isolated and analysed alone (Table 2). There are enough measurements here (3875) to permit a grouping of the deviation angles within 5° intervals. Two major features of these selected results (Fig. 3) are the same as in many of the single runs of twenty-five measurements and in the total data. These are: (1) the marked preference for a 90° orientation relative to the polarization plane; and (2) the small upturn in the frequency at 0° as noted in the 150° run of Fig. 2.

In addition, a new feature is made obvious by Fig. 3. This is the rather gradual slope upwards of the frequency curve to a maximum in the 85–90° group. This means that either the accuracy or the persistence of the mysid's orientation with reference to the plane of polarization is not high. To analyse this further, one must be able to differentiate between these two contributing factors, both tending to reduce the sharpness of the average frequency distribution curve. Some experimental data obtained on the persistence factor is presented below. By inference the remainder of the broad spread of orientation angles must be primarily determined by the precision of the response.

Whatever this precision of alignment may be, we have already seen that in the data as a whole there is great variability in the occurrence (*v.* non-occurrence) of orientated response. Thus some individual mysids obviously orientate largely by this visual cue, others may not do so in the experimental situation prevailing; even in a single animal the influence of the polarization plane may vary from time to time. An indication of the usual duration of a period of consistent response is obtained when the data for a single animal are analysed by runs. For example, one series of thirty runs had twenty-two which showed a significant preference for the 61–90° sector. Of the remainder five had a random distribution and three had a bias for angles between 31 and 60°.

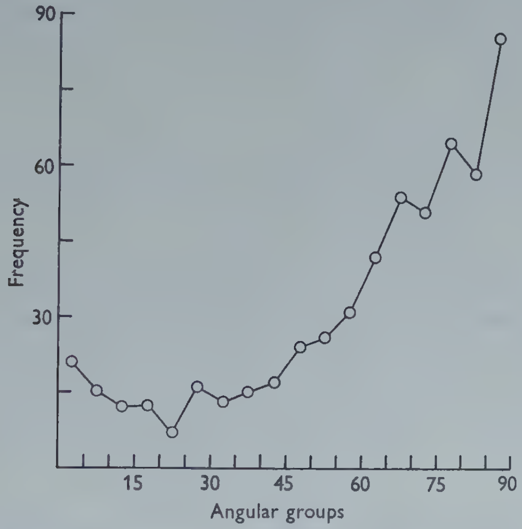

Fig. 4. Swimming orientation of *Mysidium* relative to the polarization plane in a selected series of twenty-two runs plotted with deviation angles grouped in 5° intervals. Out of thirty consecutive runs these twenty-two showed a significant preference for swimming perpendicular to the **e** vector, the remainder did not. Except that the random component of the over-all results is naturally reduced by this selection the relations are essentially similar to those shown by the whole data (Table 2 and Fig. 3).

An even more detailed idea of the accuracy of orientation is obtained if only these twenty-two runs showing the 90° bias are examined (Fig. 4). The randomness of the graph is, of course, reduced by this selection and the preponderance of polarization responses sharpened (Table 2). But the lesser peak around 0° is still present, indicating that even when the average orientation is most favourable for swimming across the plane of the **e** vector, this individual tended to orientate parallel to it some of the time. Otherwise this more selected frequency curve is essentially similar to the summed distributions.

In the sole exception to the general rule for set averages, swimming parallel to the plane was obtained in five sets measured in one animal in a single experimental session (Fig. 5). The χ^2 test applied to this data gave the following results:

Groupings of deviation angles	χ^2	Degrees of freedom	P
For 15° intervals	8·5	4	*c.* 0·70
For 30° intervals	8·0	2	0·02

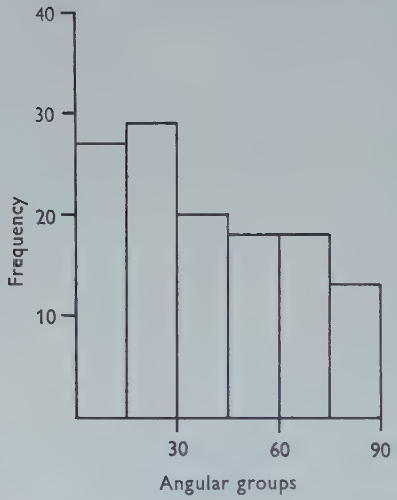

Fig. 5. Exceptional **e** vector orientation observed in a single *Mysidium* during five sets measured in one session. The 15° group differences are not significant on the basis of the χ^2 test, but the preference for near-parallel orientation is significant if 30° intervals are considered instead. This same animal gave the more usual perpendicular orientation for a long period on the previous day (Fig. 6).

Thus the tendency for swimming in the plane may be taken as significant for the 30° groupings of angles, but in the more rigorous 15° analysis this was not statistically sustained.

On the previous day this same individual *Mysidium* had shown the longest period of consistent polarized light behaviour noted in all these experiments. At that earlier time it behaved like the majority of other specimens and swam mainly perpendicular to the plane of polarization for 3 hr. Sample control runs separated by 75 min. of swimming under various other conditions confirmed the essential stability of its orientation pattern during this period (Fig. 6).

From these measurements it may also be concluded that fatigue does not have any significant effect in a 3 hr. period of response, although fluctuations in the random component of orientation are clearly shown by the three sets of histograms. Comparison of the results on the 2 days quantitatively demonstrates the sort of changes in reaction pattern which may occur in a single *Mysidium* and must usually be present to some degree in a population of individuals orientating to polarized light.

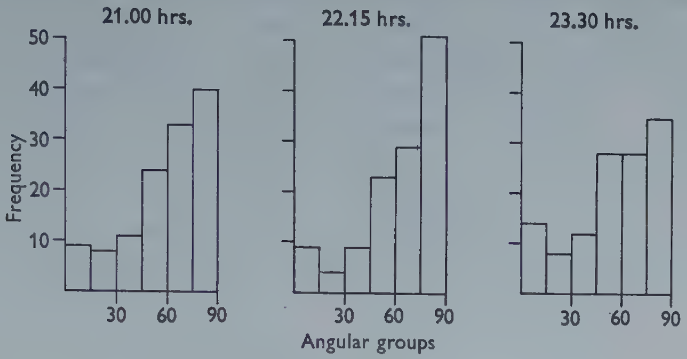

Fig. 6. Orientation to plane polarized light by the individual which, on the following day, gave the results of Fig. 5. The three sets of data were taken under identical conditions at intervals of 75 min. Between these times other tests were run. The results demonstrate the basic stability of the mysid's reaction pattern over a 3 hr. experimental period.

To check whether significant response changes of this kind were occurring within the much shorter time intervals occupied by runs of measurements with one individual, each orientation was numbered serially for the run throughout seven sets of data. Grouping these into 25 sec. intervals gives the following totals:

Time interval during 2 min. run	1–25 sec.	26–50 sec.	51–75 sec.	76–100 sec.	101–125 sec.
No. of deviation angles within the 61–90° interval	159	157	145	152	154

No indication is given here that either the animal's preference or accuracy of orientation was changing systematically or significantly within the 2 min. period of the separate runs of twenty-five observations. Under certain conditions, however, a change in some stimulus parameter after one run does influence the outcome of the next run. This occurred, for example, when balanced series of measurements were made at different intensities and wavelengths. (The data here comprise the same sequence of fifty-five runs also mentioned on p. 353.)

If the frequency distributions for ten runs made just *before* changes in I or λ are compared with runs immediately *after* such changes, an interesting difference is found. Before the light is altered the orientation pattern is characteristic of that found over long periods when only the polarization plane is changed; afterwards, the random element is much greater. This suggests that polarized light orientation requires at some level within the mysid a process akin to light- or dark-adaptation before a steady state is reached.

The summarizing description of our experimental results so far presented proves clearly that *Mysidium* can orientate its swimming in relation to a vertical beam of linearly polarized light and does so mainly at right angles to the **e** vector plane. However, the detailed data illuminate a number of further points. Of prime importance were the tests made to increase the certainty with which the general conclusions may be accepted. In some of the control experiments paired runs were made with and without the light being polarized. In others an extra diaphragm, already described on p. 344, was used to exclude more thoroughly the possibility of spurious effects introduced by the experimental cell into the polarized light beam.

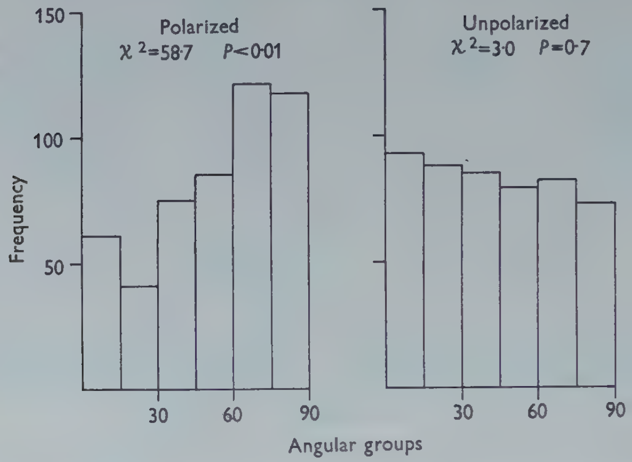

Fig. 7. Comparison of the swimming orientation of *Mysidium* in balanced runs with polarized and unpolarized light beams. Highly significant bias for orientation at right angles to the **e** vector plane is demonstrated in polarized light and no significant preference in unpolarized light.

Two double sets with two individuals were run to compare swimming behaviour in polarized and unpolarized light under otherwise identical conditions. This was accomplished by placing a second depolarizing filter made of waxed paper in the beam either above or below the Polaroid. The first position provided completely linearly polarized light, the second completely unpolarized light of the same intensity and pattern of illumination. By alternately making the polarized run the first or second member of a pair of runs for each polarization plane any persistent effect of the previous exposure on the mysid's behaviour was minimized. In this manner 500 orientation measurements were obtained with polarized and 500 with unpolarized light.

As before, the polarized sets showed a highly significant bias for swimming at right angles to the plane (Fig. 7). The χ^2 results are shown in the figure. The unpolarized sets showed a slight trend towards a maximum at 0°, but this was not statistically significant and must be considered chance variation. Therefore these control data strongly support the general conclusion that the polarization pattern really is the significant parameter here. An interesting secondary point appears in the data for the first mysid in these pairs of sets, namely that the animal ranged less about the cell in the unpolarized light than when it was orientating to the polarization. If this is a real difference, it may be compared descriptively to the apparent photokinetic effect of polarized light reported by Verkhovskaya (1940).

To re-check the possible disturbing effects of reflexion from the walls of the experimental cell, three pairs of sets were measured each with a different animal. This was done with balanced runs with and without the diaphragm (already described) completely shading the vessel's walls. The combined results for 750 readings with the diaphragm and 750 without show that this extra shielding did not significantly alter the outcome of the experiment. In both cases swimming was predominantly in the 61–90° sector, even though the random element, indicating lack of polarized light response, was rather high in these data (Fig. 8). This control result should allay any residual qualms about the presence of significant optical artifacts in the system.

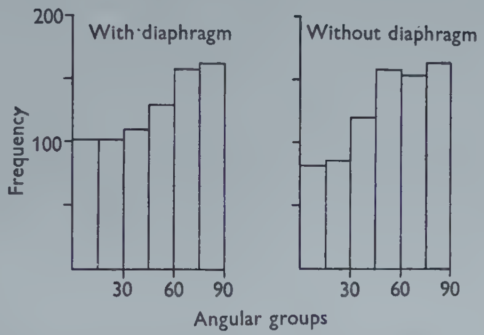

Fig. 8. Comparison of the swimming orientation of *Mysidium* to polarized light in balanced runs with and without an extra diaphragm shielding the experimental cell walls from the light beam. The extra shielding is seen not to alter the outcome, proving that even without this diaphragm, the animal is probably not reacting to artifacts reflected from the cell walls.

Finally, some of the experiments explored the effects of parameters which could provide further clues as to the sensory mechanism involved in the results described above. By means of Wratten 'neutral' filters transmitting 10 and 1%, as well as two interference filters with transmission maxima at 440 and 660 mμ, both intensity and λ of the polarized light were varied in a sequence of fifty-five runs. In one set carried out with each of these plus an initial and terminal control with full unaltered intensity, significant predominance of 90° orientation to the polarization plane occurred in every case (Fig. 9). Decreasing the intensity of the light, and perhaps also restricting the wavelength particularly to the red, caused an increase in the random element in the responses. The changes in intensity did not change the dominance of the perpendicular as compared with the parallel orientation to the plane as they have been reported to do in *Drosophila* (Stephens, Fingerman & Brown, 1953). Further experiments of the sort here described need to be done using equal spectral energies and filters actually known to be neutral for *Mysidium* whose wavelength sensitivity has not yet been determined.

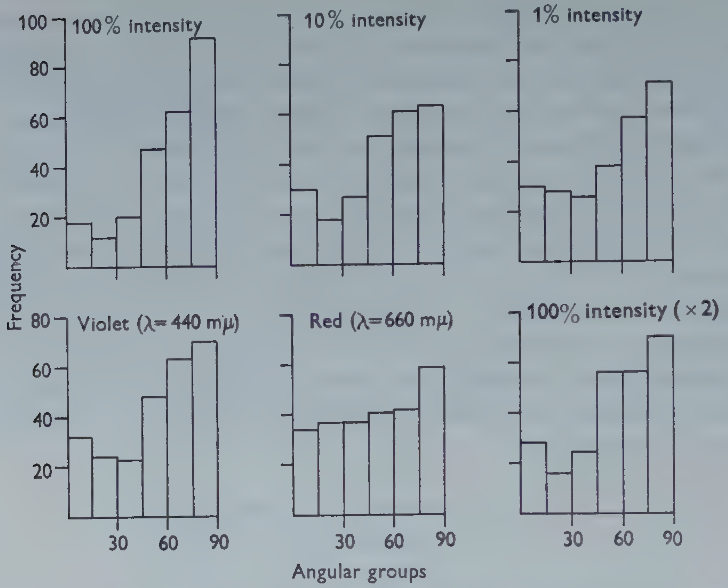

Fig. 9. Orientation of *Mysidium* in a series of sets with light passed through Wratten 'neutral' filters and narrow band interference filters. In all cases the predominance of the 90° orientation plane is clear. Decreasing the light intensity and perhaps also restricting its wavelength, particularly in the red, merely increases the random element in the swimming orientation. The final 100% intensity test was a half set and the numbers are shown here doubled.

Discussion

The responses of *Mysidium* to a vertical beam of linearly polarized light raise some interesting questions. Most of these cannot be answered definitely in the light of the present research; some, however, can be illuminated by correlation with other facts already known.

Perhaps the point of primary interest to the physiologist is whether the data provide clues to the sensory mechanism of polarized light perception. The type of reaction pattern shown by *Mysidium*, while differing in detail, is qualitatively rather similar to those found in other arthropods. Thus in a vertical beam of linearly polarized light *Daphnia*, many other species of Cladocera, and a water mite move at right angles to the plane (Baylor & Smith, 1953; Eckert, 1953). The isopods *Porcellio* and *Oniscus* orientate either parallel or perpendicular to the **e** vector (Birukow, 1956, personal communication). Among insects *Drosophila* aligns its longitudinal axis parallel to the **e** vector at high light intensities, but either perpendicular or parallel at low intensities (Stephens, Fingerman & Brown, 1953). Multiple choice is apparently available for the beetle, *Geotrupes* (Birukow, 1954). Here orientations parallel, perpendicular *and* oblique (45° and 135°) were preferred in this order. The orientation of arthropods to polarized light in the sky is a menotaxis and hence more complicated than these responses to Polaroid sheets.

On the basis of the laboratory studies cited, *Daphnia* and *Drosophila* have been claimed to use a refraction-reflexion means of polarized light analysis. According to this hypothesis many ommatidia would be involved in the sensory part of the mechanism. This is consistent with the electrophysiological effects of obliquely incident light on single facets of *Limulus* compound eyes (Waterman, 1954*b*). The present data might be consistent with this if one assumes that the organism chooses axial orientations where the refraction-reflexion patterns possibly illuminating its retinas are symmetrical. Birukow's interpretation of his *Geotrupes* results, including alignment at 45° angles to the **e** vector, does not, however, agree with this. Instead, his explanation is derived from the pattern of eight retinula cells in a single ommatidium and follows the hypothesis of Autrum & Stumpf (1950). The latter assume that each single retinula cell is a polarized light analyser, and hence one ommatidium could measure the plane in contrast to the many required by the refraction-reflexion theory. Autrum's hypothesis has been given considerable support from the electron microscopy and polarization optics of insect eyes (Fernandez-Moran, 1956; Stockhammer, 1956). But direct evidence is lacking.

In relation to animal navigation, probably the most interesting question arising from the present work relates to the observed variability of the polarized light response. This was expressed in the different effects of polarized light on various individuals and also on the same individual at different times, a variability reminiscent of much work on taxes and simple behaviour patterns generally. Extensive evidence proves that various sensory inputs, as well as certain metabolic factors, can interact strongly before a motor act occurs (Birukow, 1954; Halldal, 1957). Thus the afferent data from a single receptor system, such as the visual, can be thought of as encountering a 'central mood' which may modify, suppress or even reverse the output that might otherwise occur under the simplest conditions. Important quantitative study of such phenomena has been made in teleosts by von Holst (1950). Nevertheless, little is known of the mechanisms involved. From a general ethological point of view *Mysidium* is obviously a visually alert animal. For this reason analytical study of the problem of its attentiveness to polarized light patterns would be both interesting and worth while.

A further important component of the behavioural variability in question arises from the inaccuracy of the polarized light orientation. Even when the effect of randomly swimming specimens is minimized (Fig. 4) there is still a broad spread of observations through all possible angles. Less than 40% of the measurements showed orientation within 15° of the perpendicular to the **e** vector. Several explanations are possible for such lack of precision. In the first place one might conclude that sensory discrimination for polarized light is actually as inaccurate as this. No electro-physiological or behavioural data are yet available on the minimal discriminable change in **e** vector position in any animal. The only relevant electrical measurements (on *Limulus*) would suggest that angular sensitivity to the plane was not great in the single optic units to which tests were limited (Waterman, 1950, 1954*b*). Behaviourally other arthropods, like *Mysidium*, show rather inaccurate alignment to the polarization pattern, and the data are subject to the same limitation of interpretation as the present work. On the other hand we know that sensitivity to angular displacement may be incredibly good in some compound eyes at least

(Hassenstein and Reichardt, 1956; Lindauer, 1957), and much could hinge on the mechanism of polarized light perception.

On the other hand, apparent, rather than real, lack of visual precision would result from the occurrence of more than one stable orientation relative to a given polarization plane. Obviously the minor peak in our data at 0° is such a secondary stable axial plane. Any additional such tendencies, even if not statistically significant as peaks, could increase the apparent randomness of the results as in Birukow's experiments on *Geotrupes* (1954).

Also in our work the small size of the experimental cell forced the actively swimming mysid to turn round frequently. This may have interfered with the setting of an accurate course relative to the plane. On the other hand, the frequent turning forced the animal continually to reset the direction of its swimming so the data perhaps comprise a large number of orientation judgements rather than just one or a few initial ones. In this connexion the field experiments are of interest because such narrow and artificial confines are not involved.

FIELD EXPERIMENTS ON *PALAEMON*

Organism

The problem at hand clearly requires field studies of orientated behaviour in addition to laboratory experiments of the sort described above. One might hope to find, in aquatic forms, responses to underwater polarization patterns comparable with the orientation to sky polarization observed in terrestrial forms, e.g. by von Frisch & Lindauer (1954), Wellington (1955), and Pardi (1956). To this end a search was made for polarization influences on marine plankton organisms observed underwater as in previous work elsewhere (Bainbridge, 1952). But the populations of appropriate animals proved to be so sparse in Bermuda that profitable observations were rare. Consequently, the behaviour of a number of crustaceans was studied when they were released far enough from the shore and the bottom for these not to serve as landmarks.

Unfortunately the mysid used in the laboratory experiments proved to be unsuitable for the field work because it merely swam downward when released. However, some caridean decapods were found to swim away from such release points in an apparently directed manner. Relatively straight-line courses were not infrequent and sometimes were maintained for appreciable distances. As a result, this behaviour seemed promising for our purpose, and a series of releases were studied quantitatively in search of the directional clues involved. In this work adult *Palaemon northropi* (Rankin), a common littoral prawn, was utilized. The length of these animals was 25–50 mm. It was equally unfortunate that *Palaemon* proved unsuitable for laboratory experiments like those carried out on *Mysidium*. In a confined space the decapods' swimming was largely directed by contact with the walls of the experimental cell and not at all obviously by the light pattern.

Procedure

The site chosen for this field work was off the eastern shore of Hall's Island in Harrington Sound, Bermuda. A flat straight shore here falls steeply into deep water. Hence observations could be made reasonably close to the shore but quite out of sight of the bottom. At this point the Sound, an almost completely enclosed body of sea water about 3 km. across, is sheltered from large waves and appreciable tidal currents.

Three observers were required to record the prawn's path after release. One of these acted as a diver, wearing an Aqualung compressed-air breathing apparatus. He would swim out 30–40 m. with a closed jar containing a few healthy specimens of *Palaemon*. At a pre-arranged signal he dived into a depth of 3–5 m. and released one prawn while holding the jar above his head. Under these circumstances the animal would often circle and swim up to within 0·5 m. or less of the surface and set off on a horizontal course. The diver then followed the prawn at a discreet distance (1·5–3 m.) and for as long as possible.

The objection that in this situation the animal was in reality swimming to escape the diver is quite unfounded. On the contrary, the greatest skill had to be exercised to prevent the prawns from doing precisely the reverse. If when first released, the animal noticed the diver, it would swim to him and settle tenaciously on his person. Similarly, while following, if the diver strayed too far from the line directly behind the *Palaemon*, it would at once turn and swim to him. Most of the unsatisfactory runs resulted from this effect. The end of a run occurred generally because the animal was lost to sight as a result of the diver keeping too far behind to avoid detection by the crustacean. Meanwhile, the two other observers were stationed on the shore at water-level a measured distance apart. With sextants and using some prominent object on the distant shore as a reference point, they took sights at half-minute intervals on either the diver's hand which was held out of the water if he was near the surface, or on the patch of bubbles from his breathing. When these pairs of bearings are plotted on a large sheet of graph paper on which the base-line and reference point are recorded, then successive positions of the diver may be accurately plotted. The line connecting these points closely approximates to the animal's course.

If the courses were straight, a single compass reading could be readily given for each run. But the animal's paths were generally somewhat irregular. In analysing the records the choice therefore of only the straightest piece of the run would necessitate arbitrary selection. Similarly, the alternative of summing the deviations of each successive half-minute of course would have its drawbacks. It would, for example, be less accurate than the method used and would also unduly weight the apparently random circling at the beginning of each release. Consequently, it was decided to connect the first observation with the last by a straight line and call this the 'resultant course'. This was rarely the exact line followed by the animal but it appears to be the most impartial solution to this problem.

As the most plausible clue for the animal's sense of direction, the bearing of the sun was recorded during these observations. Measurements were generally made only when the sun was shining or else under carefully noted meteorological conditions. Previous quantitative determination of the close correlation between the sun's position and the underwater polarization pattern made it unnecessary to redetermine this potential aid to orientation (Waterman & Westell, 1956).

Results

The main body of these experiments comprises sixty-one releases of *Palaemon* made on four different days during 2 weeks of August 1956. Of these, forty-one were successfully followed and gave enough points to plot a course. To provide some basic familiarity with these results, four of the courses will be presented in detail (Fig. 10).

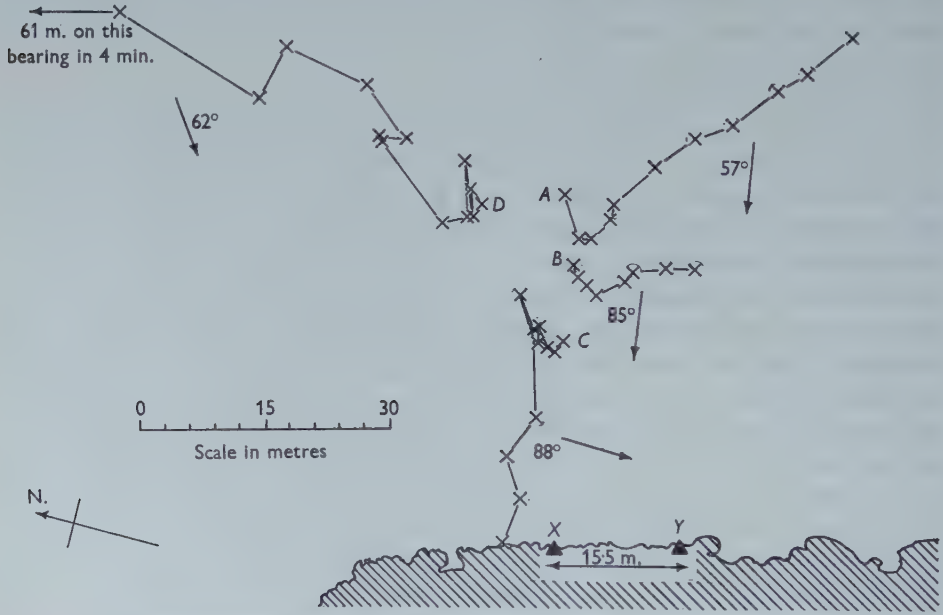

Fig. 10. Four representative courses swum by *Palaemon* released in quiet water too far from the shore or bottom for visual contact. A diver and two shore observers at *X* and *Y* worked together to fix the animal's position every 30 sec. during the run. The arrows beside the course lines indicate the bearing of the sun for each. Like all successful field data (41 cases), these runs show a predominance of courses swum at right angles to the sun's bearing, indicating that either a direct or indirect sun compass is involved.

The run represented by line *A* started in the direction of the sun, but the animal quickly veered through a half circle about 3 m. in radius and then set off on a fairly straight course for about 4 min. during which time it covered 37 m. The angle between the resultant course and the sun's azimuth was 57°. During this period of swimming the sun was clearly visible. The animal represented by line *B* started similarly by circling from the direction of the sun. It eventually set a course whose resultant made an angle of 85° with the sun's azimuth. It also swam for 4 min. before being lost but covered a distance of only 17 m. from the starting-point. The sky was again clear at this time.

Table 3. *Summary of* Palaemon *navigation observations*

Release no.	Distance swum in metres	Course in degrees E. of N.	Sun's azimuth in degrees E. of N.	Deviation of course from sun in degrees
16 Aug.				
1	34·8	127	240	67
2	7·0	119	245	54
3	19·5	179	250	71
4	16·8	202	255	53
5	27·2	204	260	56
6	4·3	324	264	60
22 Aug.				
1	7·6	117	173	56
2	4·0	22	180	22
3	6·1	80	190	70
4	3·4	16	193	3
28 Aug.				
1	63·5	161	247	86
2	29·6	153	252	81
3	49·3	121	254	47
4	25·3	156	259	77
5	14·0	146	260	66
6	41·5	138	261	57
7	22·9	157	262	75
8	6·4	156	262	74
9	15·2	168	263	85
10	29·0	160	264	76
11	6·1	141	265	56
12	6·1	121	267	34
13	2·4	103	267	16
14	11·0	169	268	81
29 Aug.				
1	25·9	242	150	88
2	51·8	81	166	85
3	23·5	67	176	71
4	25·9	271	183	88
5	6·1	315	193	58
6	13·7	28	195	13
7	7·0	258	207	29
8	44·2	345	207	42
9	13·1	230	212	18
10	8·5	340	215	55
11	23·5	13	217	24
12	5·5	293	221	72
13	8·5	324	233	89
14	13·7	3	234	51
15	110·0	353	235	62
16	41·2	41	243	22
17	119·0	342	244	82

The animal of line *C* was a female; it moved backwards and forwards and around somewhat erratically for about 2·5 min. before setting off on a relatively straight run which carried it 33 m., in 2.5 min., to the shore. The resultant course was at 88° to the sun's azimuth. The sky was partly cloudy during this period. Finally *D* represents an animal which again moved erratically for the first 2 min. but then set off on a course which, with a few deviations, took it 110 m. in 8 min. The angle between this resultant course and the azimuth of the sun was 62°. At this time the sky was partly covered by cloud, and there was a thin high haze.

Turning now to the results of all forty-one successful runs, these generally followed a common pattern. Usually after some circling and random movement but occasionally at once, the animal would set off on a fixed bearing. It then continued on this course with some deviation to left and right, but rarely turning completely, until lost by the diver. The mean length of the forty-one courses followed is 24·5 m., the maximum 119 m. and the minimum 2·4 m. The pertinent numerical data are summarized in Table 3. The last column shows that a preponderance of the resultant courses make angles close to 90° with the sun's bearing.

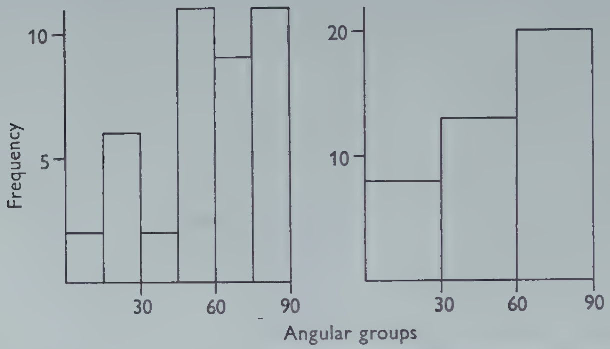

Fig. 11. Deviation angles between the sun's bearing and the resultant courses followed in forty-one releases of *Palaemon* in quiet open water. Preference shown for swimming at right angles to the sun's direction is statistically significant for both the 30° and 15° interval groupings analysed. These graphs of *Palaemon's* field behaviour are strikingly similar to those showing the polarized light orientation of *Mysidium* in the laboratory (e.g. Fig. 2A).

These deviation angles have been analysed, as in the mysid laboratory experiments, by grouping them in 15° and 30° intervals (Fig. 11). Although the numbers are hardly sufficient for the 15° grouping to be strictly regular, both of these distributions may be counted as significant on the basis of χ^2 tests:

Groupings of deviation angles	χ^2	Degrees of freedom	P
For 15° intervals	12·4	5	c. 0·04
For 30° intervals	6·1	2	<0·05

From this one can conclude that the courses followed by these prawns were significantly correlated with the sun's bearing and ran generally perpendicular to it. But the possibility of other environmental influences remains. Some water movements must have been taking place, perhaps partly from tidal action but mostly wind driven. Conceivably these or the resultant pattern of waves on the surface could have been orientating clues. The wind, however, varied from day to day; on 28 August it was fresh and from the south, blowing in a direction opposite to most of the migrations on that day; on 29 August there was little wind. Similarly, the shore line and the bottom might possibly afford visual clues to the crustacean not apparent to the diver who was quite disorientated in the experimental region except for the sun and mnemonic assistance. But if there were such a pattern, it would be fixed, and yet the runs show significant correlation with the sun which ranged through more than 90° of arc during the time of various observations. It would thus appear just to conclude that *Palaemon* can use the sun either directly or indirectly as a celestial compass.

In addition to the main results the data provide information on one or two points of further interest. For example, they permit the speed of swimming of these crustaceans in nature to be calculated. A common rate measured along the resultant course is that for release 11 on 28 August which is 9 m./min. The maximum swimming velocity was measured in release 6 of 28 August. Here the *Palaemon* swam 34·5 m. in 2 min., which is a rate of about 0·3 m./sec. or nearly 1 km./hr. Considering that the specimen's length approximated to 0·038 m. this seems a formidable accomplishment.

A common aspect of the prawn's swimming pattern is of interest in relation to the behaviour of plankton. This is the fact that although the animal's course was straight in a horizontal plane, it often was sinusoidal in a vertical plane. The wavelength of this motion was 2–3 times its amplitude of 20–30 cm. It appears quite likely that this is a form of the 'hop and sink' behaviour by which vertically swimming plankton animals keep an optimal position in the water mass (Hardy & Bainbridge, 1954). Like them the *Palaemon* could have been sampling deeper and shallower layers and by reacting to alternating changes in factors such as light or pressure maintaining its level in the water. The distinction is that the prawn has a strong horizontal component superimposed upon its vertical one.

Discussion

The ecological consequences of such orientated horizontal swimming in nature could of course be considerable. Adaptive evolution of the mechanism might be expected if, for example, it assisted herbivorous animals in finding fresh areas of the phytoplankton, which is so patchy in distribution (Bainbridge, 1957). The whole mechanism could in fact fulfil the need postulated by Bainbridge (1953) for a system imposing directionality on the general horizontal movements of such Crustacea in the sea. Breeding and other migrations (Sutcliffe, 1953) might also be assisted by directed movements of this sort.

The resemblance between the caridean's orientation relative to the sun's bearing in the field studies (Fig. 11) and the mysid's swimming relative to the polarization plane in the laboratory (Fig. 2A) is extraordinary. It may be merely coincidental, but yet again may derive from a comparable sensory and response mechanism in the two cases. If the *Palaemon* was reacting to the underwater polarization pattern rather than directly to the apparent position of the sun itself, the question of which part of the whole pattern is involved raises an interesting point. Since underwater polarization is generally in planes at right angles to the submarine direction of the sun's rays, the animals would have been orientating and swimming parallel to the **e** vector observed both directly beneath them and overhead. If so, this is different from the usual polarized light response of *Mysidium* observed in the laboratory, where perpendicular alignment predominated.

The task of differentiating between a sun compass and a polarized light compass underwater is a challenging one. It would require essentially the discovery of an orientated behaviour pattern which will continue to appear even when the animal is shaded from the direct, but not the scattered, rays of the sun. This, of course, has been the basis for proving the use of sky polarization by terrestrial arthropods. An additional demonstration that a polarizing film interposed in the animal's line of sight will change its orientation as the **e** vector is rotated, would support and complement the evidence of the first criterion. Further field work is planned to extend the results herein described and to test these crucial points. At the same time the further development of the quantitative data from the laboratory experiments will be pursued in an effort to analyse the effective parameters of the polarized light stimulus. So far these are practically unknown.

SUMMARY

1. Quantitative experiments are described, designed to test the hypothesis that underwater polarized light is used for orientation by aquatic animals as that of the blue sky is by terrestrial arthropods.

2. Studied in the laboratory the littoral mysid, *Mysidium gracile*, was found capable of orientation relative to the **e** vector in a vertical beam of linearly polarized light.

3. Statistically significant preference was shown by this animal for aligning its longitudinal axis at right angles to the plane of polarization.

4. A secondary slight preference was shown for swimming parallel with the plane and the degree of random orientation was often high (40% mean).

5. The littoral prawn, *Palaemon northropi*, when released in quiet water far enough from the bottom and the shore to prevent visual contact, was found to swim on relatively straight horizontal courses for appreciable distances in forty-one out of sixty-one tests.

6. The mean length of these runs was 24·5 m., the maximum 119 m.; swimming velocities up to about 1 km./hr. were measured.

7. The sun's position seems to be the only directional clue not controlled in these tests, and the general direction of the runs is significantly correlated with the direction of the sun, being at right angles to it.

8. It is thus concluded that this animal can use the sun, either directly or through the polarization pattern, as a celestial compass.

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STUDIES ON POSSIBLE SEROLOGICAL BLOCKS TO SPECIES HYBRIDIZATION IN POULTRY

By MARGARET RYLE

School of Agriculture, University of Cambridge[*]

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INTRODUCTION

A number of attempts at crossing various species of birds have proved more or less successful according to the direction of the cross (e.g. Painter & Cole, 1943). Marked differences of fertility resulting from reciprocal matings have been observed by Haig Thomas & Huxley (1927), Warren & Scott (1935), Quinn, Burrows & Byerly (1937) and Ryle & Simonsen (1956). These authors, and Owen (1941), also observed differences of viability between the hybrid embryos resulting from such reciprocal crosses. The latter worker, in agreement with Haig Thomas & Huxley (1927) and Shaklee & Knox (1954), found great variations between individual hens in respect of the fertility of their eggs after insemination with semen of another species. The environment for the sperms within the oviduct and ovum, and that for its chromosomes within the zygote cytoplasm, are dependent on the constitution of the female; so also is the environment provided for the embryo. Reciprocal differences in fertility and embryonic viability indicate that one or more of these environmental factors are of considerable importance. Differences between the fertility of individual hens could be due to conditions external to the egg which, with greater or less efficiency, prevent the sperm from reaching the ovum nucleus. Similarly, where only a small proportion of the embryos in a given cross develop to a late stage, they might owe their success to rare, exceptionally favourable conditions derived from particular hens.

There are probably many physiological factors which can block hybrid fertilization and development. Among them natural (normal) antibodies may be of some importance. Simonsen has pointed out (personal communication) that the normal anti-chicken-erythrocyte heteroagglutinin titre of turkey serum is much lower than the normal anti-turkey-erythrocyte heteroagglutinin titre of chicken serum. This parallels the different results of reciprocal matings of the two species, the fowl ♂ × turkey ♀ cross giving greater fertility and embryonic viability than the turkey ♂ × fowl ♀. Some major blood-group antigens occur on mammalian spermatozoa (Landsteiner & Levine, 1926; Yamakami, 1926; Docton, Ferguson, Lazear & Ely, 1952), which also have species-specific antigens (Mudd & Mudd, 1929; Henle, 1938). Possibly avian spermatozoa share with erythrocytes species-specific antigens susceptible to the normal agglutinins of other species. Antibodies may pass in some way into the lumen of the avian oviduct, and there agglutinate or otherwise damage heterologous spermatozoa. If so, differences of normal heteroagglutinin titre could contribute to individual variations in fertility as well as to the differences shown by reciprocal matings. Within the egg, maternal heteroagglutinin could react with antigens of paternal origin as these appear in the hybrid embryo, and so interrupt normal development. Numerous authors (cf. Cottrall, 1952; Buxton, 1952) have published evidence on the transfer of immune antibodies from the hen via the egg yolk to the embryonic or chick serum. It seems likely that natural antibodies are also passed on in this way and that, when hybrid fertilization occurs in the presence of a relatively high titre of antibody reacting with antigens of the paternal species, the probability of normal embryonic development is less than in the presence of a low titre. One may compare the known cases of haemolytic jaundice, both natural and artificially induced (e.g. Briles, 1948), due to serological incompatibility of dam and foetus within a species. Again differences of titre between species and among individual hens would contribute to differences in embryonic viability. With these considerations in mind the series of tests described below were undertaken.

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[*] Present address: c/o Department of Zoology, University of Melbourne, Australia.

MATERIALS AND METHODS

Citrated plasma—1 part 3·5% trisodium citrate: 4 parts whole blood—was used for titrations, which were normally carried out on the day of collection. In the few cases where this was not possible, the samples were stored in the freezing compartment of the refrigerator for not more than 14 days. Twofold serial dilutions, with two drops of fluid at each dilution, were set up; one drop of the appropriate cell suspension was then added to each tube. For determining the heteroagglutinin titre of yolk the following technique proved satisfactory: part of the egg shell and shell membranes near the widest part of the egg were removed and most of the albumen was carefully poured off. The vitelline membrane was punctured with the tip of the syringe used to withdraw a sample of yolk. This sample was added to an equal volume of 2% saline in a centrifuge tube, which was then shaken vigorously until the yolk and saline were thoroughly mixed. Prolonged centrifugation threw down most of the flocculent material in the mixture, but it proved advantageous to allow the supernatant to stand overnight in the refrigerator to clear completely. For titrations twofold serial dilutions of the yolk extracts were prepared, using 2% saline. When setting up serial dilutions of several yolks for comparative titrations, the pipette was rinsed with 10% saline, before final washings in 2%, to remove traces of yolk sticking to the glass. The titres of some test extracts in 2% saline were identical with 1:1 mixtures of the same yolks in 1% saline. The latter, however, were always cloudy as the flocculent material could not be thrown down; it was therefore impossible to examine the cell desposits in them macroscopically. Neither chicken nor turkey erythrocytes appeared to be damaged by 2% saline, although 3% or higher concentrations caused them to clump together. Nevertheless, yolk tubes in which only a very few pairs of agglutinated cells were observed have been recorded throughout as 'doubtful'. Undiluted yolk is too viscous for satisfactory agglutination tests, so no dilutions less than 1/2 were used.

Erythrocyte suspensions were prepared by washing freshly collected, packed cells, depositing them by centrifugation and preparing a 2% suspension, in 1% saline for plasma titrations and 2% saline for yolk. After adding the erythrocyte suspension to the plasma or yolk dilutions the tubes were normally left for 1 hr. at room temperature before the sediment was examined; the titre of a given plasma or yolk sample is, as usual, defined as the reciprocal of the highest dilution in which agglutinated cells were detected. Considerable difficulty was encountered in the work with spermatozoa because they tend to agglutinate in the plasma of their own species and sometimes even in that of the same bird. In order to overcome such non-specific agglutination various fluids were tested for washing and suspending them, and serum was used in place of citrated plasma in some preliminary trials. Smith (1949) found Baker's (1932) fluid suitable for agglutination tests with mammalian sperm. Of the fluids tested in the present work, it was also the most satisfactory for washing and suspending avian sperm. Its formula is: glucose, 3·0 g.; $Na_2HPO_4.12H_2O$, 0·6 g.; NaCl, 0·2 g.; KH_2PO_4, 0·01 g.; water, 100 ml. Serial dilutions in Baker's fluid of the plasmas to be titrated were set up before the semen was collected, and the agglutination tubes were immersed in cold tap water (10° C. approx.). Semen samples were collected by the method of Burrows & Quinn (1937), an apparatus being devised to facilitate collection of adequate samples from individual turkeys (Ryle, 1956). The first semen sample obtained from any bird after a long interval was discarded, since senile sperm might tend to agglutinate particularly readily, and a further sample, obtained 2 or 3 days later, was used for the agglutination tests. The collecting apparatus was rinsed out with Baker's fluid before use and the semen mixed with a further quantity of it in small tubes immediately after collection. Mixing was carried out by vigorous shaking only, to avoid partial disintegration of any existing clumps of spermatozoa. The tubes were centrifuged slowly to throw down particles of excretory matter with associated clumped sperm. The homogeneous milky supernatant was then pipetted into fresh tubes and centrifuged more vigorously to throw down the free cells. If, however, these were packed too firmly, further clumping occurred. The sperm were again washed and centrifuged, after which a test suspension was prepared by adding approximately one drop of the loosely packed cells to 25 drops of the diluent. If not used immediately the tube containing the suspension was immersed in cold water. When titrating chicken plasma with turkey sperm, control tubes with undiluted plasma from three or more turkeys were included in the tests, as well as a control tube containing only the diluent, to check the degree of non-specific agglutination. After mixing, the agglutination tubes were allowed to stand in cold water for 45 min. before examination with a hand lens against a dull black background. No readings were made more than 3 hr. after collection of the semen. A single large pellet of spermatozoa, or coarse strands not broken by gently tapping the tube, formed in strongly agglutinating plasmas. Several medium-sized clumps or a network with moderately coarse strands indicated intermediate agglutination.

A fine network which dispersed on gentle tapping occurred in weakly agglutinating heterologous plasmas, sometimes in homologous plasmas, and even occasionally in the pure diluent. Such fine networks were regarded throughout as negative for the purpose of assessing plasma titres.

RESULTS

(1) *Variation of normal heteroagglutinin titres*

Plasma samples of seventy domestic hens of six different breeds were titrated with the erythrocytes of a Mammoth Bronze turkey. Table 1 shows the distribution of titres within each breed. It will be seen that the titres varied not only between individuals within a breed, but also between breeds. Thus the titre of sixteen of the nineteen bantams was less than 4, while only five of the twenty-four Brown Leghorns had so low a value; this difference is highly significant ($P < 1\%$). One may compare the results of Stern & Davidsohn (1954) who found that the normal anti-sheep and anti-chicken agglutinin titres varied considerably and significantly between thirteen different inbred strains of mice. One cannot of course assume here that the values are characteristic of the breeds as a whole, but only that they represent the particular strains available for the survey. Nevertheless, they suggest that variations in fertility and embryonic viability might arise from serological variations within a species. On the other hand, repeated attempts to obtain hybrid embryos from four of the hens with particularly low titres—including the bantam where no heteroagglutinin was detected—met with failure, so indicating that normal erythrocyte-agglutinating antibody is at least not always the critical factor preventing cross-fertilization. Nothing, however, is known of the incomplete, non-agglutinating antibody titres of these hens' plasmas; it is conceivable that they do not vary strictly in parallel with the complete agglutinin and that they are of more critical importance with respect to fertility.

Table 1. *Distribution of hens of different breeds according to their normal anti-turkey-erythrocyte agglutinin titres*

Breed	Total no. tested	Titre					
		<1	1	2	4	8	16
Brown Leghorn	24	—	—	5	10	7	2
Legbar	13	—	—	3	7	2	1
Polish Bantam	19	1	6	9	2	1	—
White Leghorn	7	—	—	2	5	—	—
Dorbar	6	—	—	2	3	1	—
Light Sussex	1	—	—	—	1	—	—

(2) *Agglutination of sperm by normal antibodies*

Parallel titrations of a number of chicken plasma samples were carried out with turkey erythrocyte and sperm suspensions. Seventeen of the samples were from young chickens which had only just developed the effective antibody and ten others were from adult birds; there was no obvious difference between the two groups.

The erythrocyte titres tended to be higher than the corresponding sperm ones, probably because the technique used to measure the former was more sensitive. In Table 2 the distribution of all twenty-seven plasmas is set out according to their

Table 2. *Distribution of fowl plasmas according to their titres as measured with turkey spermatozoa and erythrocytes*

Titre with sperm	Titre with erythrocytes					
	1	2	4	8	16	32
16	—	—	—	—	—	1
8	—	—	—	1	—	—
4	—	—	3	1	1	—
2	1	3	3	6	—	1
1	1	3	—	1	1	—

Table 3. *Titres, with erythrocytes and spermatozoa of the same turkey, of various fowl plasmas before and after absorption with each type of cell*

Plasma no.	Plasma titres					
	Before absorption		After absorption with erythrocytes		After absorption with spermatozoa	
	Sperm*	Eryth.*	Sperm*	Eryth.*	Sperm*	Eryth.*
a	2	32	< 1	< 1	—	—
b	8	8	< 1	< 1	—	—
c	—	8	—	—	—	2
d	—	2	—	—	—	2
e	—	8	—	—	—	8
f	—	16	—	—	—	8
g	—	16	—	—	—	16
h	—	4	—	—	—	4
i	—	8	—	—	—	8
j	2	8	1	—	—	—
k	4	8	< 1	—	< 1	< 1
l	2	8	1	—	< 1	< 1
m	2	4	< 1	—	< 1	< 1
n	2	4	< 1	< 1	—	—
o	2	4	< 1	< 1	< 1	< 1
p	4	4	< 1	< 1	< 1	< 1

* = Titrated with. — = no titration.

titres both with erythrocytes and with spermatozoa from the same turkey; it suggests a more or less parallel variation. If this were due to the normal hetero-agglutinin of the chicken plasma reacting with the same or closely related antigens present on both types of cell, it should be possible to remove all the anti-sperm antibody by absorbing the plasma with erythrocytes, and vice versa. Absorption tests were therefore carried out by adding packed, washed cells of one or other type to samples of chicken plasma, leaving them for a time, then re-titrating the plasma with the alternate type of cell. The results are summarized in Table 3. Absorption with erythrocytes in every case reduced the anti-sperm titre. Although absorption with spermatozoa gave contradictory results with respect to the anti-erythrocyte titre, this was probably due to the fact that the ratio of the sperm cell volume to the plasma volume during absorption of plasmas *c–i* was about half that used for plasma *k–p* and was inadequate. Unfortunately, check titrations of the absorbed plasmas with sperm suspensions were not carried out on the former samples. In general, the results indicate that turkey erythrocytes and spermatozoa share to at least a considerable extent those antigens which react with the normal complete agglutinins in chicken blood.

Plasma samples were available from only five turkeys, and of these two failed to agglutinate chicken erythrocytes even before absorption. There were indications in the remaining three of some substance which caused clumping of chicken sperm but not of red cells, and which could not be removed by absorption with the latter. This could imply that the two types of cell do not share the same antigens but that each encounters an effective antibody in turkey plasma. On the other hand, some factor in turkey plasma other than normal antibody may cause the clumping of chicken sperm. In this connexion it should be mentioned that the plasma of a small number of chickens was found to cause the clumping of cockerel sperm, although erythrocytes of the same birds were unaffected. One female of this group was normally fertile with one of the test cockerels, suggesting that the sperm-specific substance does not reduce fertility. Similarly, the sperm-specific substance in turkey plasma appears to be relatively innocuous, for the domestic fowl ♂ × turkey ♀ cross produces far more fertile eggs than its reciprocal. Further work is required to determine whether these sperm-specific substances are antibodies or some other component of the plasma not normally encountered by spermatozoa.

(3) *Isoagglutinins in relation to fertility*

In order to simulate the interspecific situation, in which the plasma of the hen agglutinates the red cells of the cock, three domestic hens were injected intravenously with 2 ml. whole citrated blood from a cock of the same breed. Samples of blood were subsequently collected at intervals from these birds and the hens' plasmas titrated with the cock's erythrocytes. Before and after immunization the three hens were inseminated twice weekly with semen from the same cock. Very few of the eggs laid before immunization, and none of those during the test period after injection were infertile; immune anti-erythrocyte agglutinin did not depress fertility. It might have been more illuminating to carry out this test with turkeys, where the spermatozoa and erythrocytes definitely have antigens in common, but this was not possible. Alternatively, the effects of anti-sperm immune agglutinins on fertility could have been investigated by immunizing the hens with suspensions of sperm instead of red cells. The results of Lamoreux (1940) suggest however, in contradiction to those of McCartney (1923), that such injections would have no effect on fertility either.

Nevertheless, while no relationship was demonstrated between red-cell *immune* isoagglutinins and fertility, the presence of *normal* isoagglutinins may be of some importance in this connexion in chickens. Some preliminary and as yet not conclusive observations on this question have been reported elsewhere (Ryle, 1956). Normal isoagglutinins probably occur in only a minority of fowls. The available data suggest that, when present, they may be associated with reduced fertility where the cock's erythrocytes carry the corresponding antigen(s). Moreover, certain contradictory features in the evidence indicate that investigation of normal *incomplete* antibodies might be profitable in this connexion as well as in relation to interspecific fertility.

(4) *Normal heteroagglutinin in yolk*

The results of preliminary tests with yolk extracts from eggs of three turkeys and eighteen chickens, using the erythrocytes of two different turkeys and two different chickens are shown in Table 4. They clearly indicate the presence of normal heteroagglutinin in yolk. Comparisons between the yolk titre and the blood titre of individual hens were next made. Citrated plasma samples were obtained from thirty-two hens selected for a wide range of heteroagglutinin titres, and also from two hens which had been injected intramuscularly at weekly intervals with whole turkey blood. The plasmas were titrated in the usual manner. Yolk extracts were prepared from fresh eggs, laid not more than 10 days before the blood samples were collected, the majority having been laid within 4 days of that date. These were titrated with cells from the same turkey as those used for the plasma samples. After standing for $1\frac{1}{2}$–2 hr. at room temperature the sediment in each plasma and yolk tube was examined microscopically. Table 5 shows the results. Clearly there

Table 4. *Agglutination of turkey and chicken erythrocytes in turkey and chicken yolks*

		Turkey cells	Chicken cells
Turkey yolks:	Positive	0	4
	Doubtful	1	2
	Negative	5	0
Chicken yolks:	Positive	25	0
	Doubtful	5	6
	Negative	6	30

Table 5. *Distribution of hens according to the anti-turkey agglutinin titre of their plasma and egg yolk*

Plasma titre	Yolk titre					
	<2	2	4	8	16	32
32	—	—	1	—	—	2*
16	—	1	2	1	—	—
8	1	2	2	3	2	—
4	2	4	2	1	—	—
2	4	3	—	—	1	—
<2	—	—	—	—	—	—

* Immunized birds.

is a fairly close correlation between the titres of the plasma and of the yolk, the latter being on the average one dilution lower than the former. This contrasts sharply with much of the published data on relative titres of immune agglutinins in yolk and blood where in general the yolk titres are very much lower than the serum titres.

(5) *The time of appearance on embryonic erythrocytes of antigens sensitive to normal heteroagglutinins*

Small batches of chickens eggs laid by hens of various breeds were set in the incubator on consecutive days. After the last batch had been incubated for 3 days, blood was collected from all the embryos. In those of 9 days' incubation or more it was usually possible, after removing a small piece of shell and making the underlying shell membrane translucent with medicinal paraffin, to take blood with a hypodermic needle directly from one of the larger allantoic vessels. In those incubated for a shorter period both shell and membrane were removed to make a large window. In 6–9-day embryos one of the larger allantoic vessels was usually drawn out over the edge of the hole, cut and allowed to bleed on to the cleaned shell surface, the drops being collected with a syringe. In 4–6-day embryos one of the larger vitelline vessels was cut *in situ* and the blood which flowed out was collected, along with a considerable quantity of yolk. In the youngest stages the whole embryo was cut across and the blood collected similarly. The syringe and needle were rinsed with 3·5% trisodium citrate before collecting the blood in all cases, and a few drops of the solution were added to the larger samples collected from the oldest embryos. The sample tubes were centrifuged or allowed to stand until the cells had settled (the latter proved more satisfactory when much yolk was present), the supernatant was removed, the cells washed with 1% saline and again thrown down by centrifugation. The washed cells were then resuspended in saline, usually as an approximately 2% suspension, but in some cases where there were few cells the dilution was greater. One drop of each cell suspension was added to a tube containing two drops of citrated normal turkey plasma. Seventeen control tubes were set up with plasma from several hens and cells of embryos ranging from 7 to 13 days' incubation. The tubes were left overnight in the lower part of the refrigerator and were examined microscopically the following day. The degree of agglutination was scored according to an arbitrary scale ranging from 1 (a very few cells clumped in very small groups) to 6 (practically all cells clumped in large masses). Where no clumped cells were seen the sample was scored as 0. There was no sign of agglutination in any of the control tubes but, as Table 6 shows, there is a sudden increase in the degree of agglutination by turkey plasma at 4–5 days' incubation, with little further increase thereafter. In fact, similar scores are obtained when cell suspensions from adult chickens are mixed with undiluted normal turkey plasma. It might be objected that the mixing of yolk with the blood of the youngest embryos during collection prevented agglutination at 3 and 4 days. However, yolk was also mixed with the cells of 5- and 6-day embryos, so it cannot account for the failure of the youngest samples to agglutinate. The onset of agglutination coincides with the beginning of the replacement of the larger primitive erythrocytes by the smaller definitive ones (Dawson, 1936), but the agglutinogen is not confined to the latter, for mixed clumps of large and small cells were observed in a number of tubes.

Table 6. *Distributions of chick embryos of various ages according to the degree of agglutination of their erythrocytes by normal turkey plasma*

Days incubation	No. of embryos	Degree of agglutination						
		0	1	2	3	4	5	6
3	4	3	1	—	—	—	—	—
4	6	4	2	—	—	—	—	—
5	5	1	—	—	2	1	1	—
6	3	—	—	2	1	—	—	—
7	5	—	—	—	2	1	1	1
8	3	—	—	1	1	—	1	—
9	3	—	—	—	1	2	—	—
10	3	—	—	—	—	2	1	—
11	3	—	—	—	1	1	1	—
12	5	—	—	—	2	3	—	—
13	4	—	—	1	—	3	—	—
14	4	—	—	—	1	2	1	—

(6) *The time of passage of normal heteroagglutinin from the yolk to the blood plasma of the embryo*

Normal anti-turkey-erythrocyte agglutinin is not produced by the chick until 4–6 weeks after hatching (Simonsen, 1955; Ryle, 1956). Buxton (1954) has demonstrated that immune antibodies, including incomplete ones, do not develop before 11 days after hatching. Before that time it can be assumed that any antibodies in the embryonic or chick plasma are of maternal origin. In order to determine the time at which normal heteroagglutinin passes from the yolk into the embryonic blood, groups of chicken eggs were set on consecutive days as in the previous experiment. The eggs were from various hens of various breeds and the anti-turkey-erythrocyte titre of their yolks probably differed considerably, but they did not include any from hens known to have very low agglutinin titres. In most cases blood was obtained direct from one of the larger allantoic vessels. In a few of the smaller embryos, where this was difficult, a vessel was drawn out over the edge of the window, cut, and allowed to bleed on to the cleaned shell, the drops of blood being collected with a syringe. The syringe was rinsed with 3·5% citrate before use in all cases. A 2% suspension of washed turkey red cells was prepared, and one drop of this was added to two drops of each of the plasma samples. The tubes were left in the refrigerator overnight before microscopic examination of the sediment. As Table 7 shows, the youngest embryo in which any agglutinin was detected was 13 days old. This is in fair agreement with Buxton's (1952) observations on the passage of immune agglutinins from the yolk to the embryonic plasma; he detected such antibodies in the embryonic plasma from 11 days' incubation. The high proportion of embryos in which no agglutinin was detected may have resulted from even greater dilution of the antibody in the embryonic fluids than in the yolk.

Table 7. *Occurrence of normal anti-turkey heteroagglutinin in blood plasma of chick embryos of various ages*

Days incubation	Total no. of embryos	Without agglutinin	With agglutinin
8	2	2	0
9	2	2	0
10	2	2	0
11	4	4	0
12	4	4	0
13	7	6	1
14	5	5	0
15	4	4	0
16	6	6	0
17	5	4	1
18	5	3	2
19	1	0	1

DISCUSSION

The above results show, first, that normal hetero-antibody titres vary considerably within a species as well as between species, a variation which might be correlated with individual differences of fertility and/or embryonic viability in interspecific crosses. With respect to the question of fertility, turkey spermatozoa share antigens with turkey erythrocytes and are agglutinated *in vitro* by the normal antibody of chicken plasma. The presence of antibody in the fowl oviduct has not, however, as yet been demonstrated, and attempts at hybridization using chickens with little or no anti-turkey-erythrocyte agglutinin were unsuccessful. Moreover, high titres of immune anti-erythrocyte isoagglutinin failed to induce intraspecific infertility in chickens. Wiener, Wexler & Hurst (1949) found that incomplete (univalent) antibodies traverse the intact human placenta more readily than complete ones; moreover, the ratio of the two types in the serum varies from one individual to another as well as from time to time (Wiener, 1951). It is possible that incomplete antibodies pass into the lumen of the avian oviduct more readily than agglutinating ones and are there able to inhibit fertilization, whether inter- or intraspecific, by damaging the sperm. The materials, antibodies or otherwise, in the plasma of turkeys and some chickens, which agglutinate the sperm of domestic cocks but not their erythrocytes, presumably cannot pass into the oviduct since they seem to have little influence on fertility. The failure to detect complete agglutinins in the plasma of certain birds may not necessarily imply the absence of incomplete antibody. Similarly, a relatively high agglutinin titre may not necessarily be correlated with a high titre of incomplete antibody. An investigation into the possible relationship between incomplete antibodies and both inter- and intraspecific fertility is indicated before final rejection of the hypothesis.

Regarding embryonic viability, it has been shown that normal anti-erythrocyte heteroagglutinin is present in egg yolk at about the same titre as in the hen's blood. It has also been shown that, in the chick embryo, the antigens which react with the normal agglutinins of turkey plasma can be detected on the erythrocytes from at least 5 days' incubation. On the other hand normal agglutinin was only detected in embryonic plasma after 13 days' incubation or more. In view of this one might tend to conclude that serological reactions could not account for early embryonic deaths. However, it should not be assumed that the relevant antigens are confined to the erythrocytes. They may be present on other tissues at even earlier stages of differentiation, when the embryo is more directly exposed to the yolk. Thus, for example, maternal antibody, by reacting with paternally derived antigens, might interfere directly with the normal morphogenetic movements of the hybrid gastrula. The results of Ebert (1950) are of great interest in relation to possible reactions between maternal antibody and the hybrid embryo. He cultivated explanted chick blastoderms *in vitro* in the presence of various anti-chicken organ sera. In low dilutions all such sera arrested development and led to general disorganization of the embryo. At intermediate dilutions differentiation was normal but no growth occurred, while at high dilutions there were no detectable effects. The recent account by Tyler & Brookbank (1956*a*, *b*) of the effects of anti-fertilization and anti-embryo sera on cell division in sea-urchin embryos is also relevant. They found that cleavage was blocked by these antisera, and in particular by anti-fertilisin, the corresponding antigen being present on the cell surfaces. With increasingly higher dilutions of the antisera, development proceeded correspondingly further before blockage, followed by cytolysis, took place. Nuclear as well as cytoplasmic division was arrested.

The majority of turkey-chicken hybrids die within the first 3 days of incubation (though a few survive and develop to about three weeks). The heteroagglutinin titre in the yolk is low and could presumably permit, by analogy with the above results, a certain degree of development even after the appearance of the reacting antigens. The relevant antigens were not detected on erythrocytes until about 5 days, but may, perhaps, be present elsewhere before that. They may even be present on the erythrocytes, but in too small a quantity or on too few cells to be detected by the simple methods used. Burke, Sullivan, Petersen & Weed (1944) showed that some antigens typical of adult fowl erythrocytes were present on the embryonic cells from about 4 days' incubation. Briles, McGibbon & Irwin (1950) found that certain fowl erythrocyte antigens could already be detected as early as 3 days, although the intensity of the agglutination reaction was very weak at this time. Similarly, Miller (1953) found that all known species-specific red-cell antigens of *Columba guinea* were present by 3 days' incubation in backcross hybrid pigeon embryos. Schechtman (1952) showed that several antigens other than those derived from the yolk could be detected in chick embryonic blood serum as early as 3 days, while Ebert (1952) found that antigens characteristic of several adult fowl tissues first appeared at that time. It is therefore not inconceivable that early hybrid embryo deaths coincide with, or follow closely upon, the appearance of some paternal species-specific antigens sensitive to the maternal heteroagglutinins. It is also possible that those embryos which survive the critical early stages may succumb when the bulk of the yolk antibody passes into the blood stream during the later stages of incubation. The present work can only suggest these possibilities; further experimentation must decide whether in fact they contribute to the difficulties of species hybridization in birds.

SUMMARY

1. It is suggested that normal heteroantibodies may contribute to the difficulties of species hybridization by damaging heterologous spermatozoa and/or by reducing hybrid embryonic viability.

2. Techniques are described for titrating yolk antibody and for agglutination tests with fowl spermatozoa.

3. Plasma samples from several breeds of chickens were titrated with turkey erythrocytes in order to study the variability of normal heteroagglutinin titres.

4. Turkey spermatozoa were agglutinated *in vitro* by chicken plasma. Absorption tests showed that the same antigens, reacting with the normal heteroagglutinin of chicken plasma, occur on turkey spermatozoa and turkey erythrocytes. Further work is required on the agglutination of chicken sperm by turkey plasma.

5. Low titres of anti-turkey-erythrocyte agglutinin in the blood of domestic hens did not promote interspecific fertility. Immunization of domestic hens with the erythrocytes of the cockerel with which they were mated did not induce infertility. It is nevertheless considered that an investigation of normal incomplete antibody in relation to inter- and intraspecific fertility might prove fruitful.

6. Normal heteroagglutinins occur in turkey and chicken yolk. The titre of anti-turkey agglutinin in chicken yolk is very close to that in the hen's plasma.

7. Antigens reacting with the normal heteroagglutinin of turkey plasma appear on chick embryo erythrocytes after 4–5 days' incubation; there seems to be little quantitative increase thereafter. They occur on both primitive and definitive erythrocytes.

8. Normal anti-turkey agglutinin derived from the egg yolk was detected in the blood plasma of a few chick embryos after 13 days' incubation or more.

9. The latter results are discussed in relation to other published work both on the effects of antisera on embryogenesis and on the ontogeny of antigenic properties.

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THE EFFECT OF LESIONS TO THE VERTICAL AND OPTIC LOBES ON TACTILE DISCRIMINATION IN *OCTOPUS*

By M. J. WELLS and J. WELLS
Department of Zoology, University of Cambridge and the Stazione Zoologica, Naples

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INTRODUCTION

Octopus has a well-developed chemotactile sense. Using the arms to examine objects touched, octopuses blinded by cutting the optic nerves can be taught to distinguish between objects that differ only chemically as well as between objects made of the same material that differ only in their non-chemical surface characteristics. They can be taught to distinguish between various naturally occurring objects such as different species of shellfish, as well as between artificial objects that differ only in the degree of irregularity of their surfaces (Wells & Wells, 1956).

From the results of these experiments and the known structure of the sensory nervous system in the arms, it can be argued that whilst the brain of *Octopus* must contain a mechanism for computing the proportion of sense organs stimulated by contact with an object, there is no need to suppose a spatial projection of the sensory field into the central nervous system, or to suppose that *Octopus* is capable of scanning objects by correlating successive sensations with proprioceptive information about the position of its suckers (Wells & Wells, 1957).

The present account is the first of a series reporting experiments designed to locate those parts of the brain of *Octopus* concerned with tactile discrimination and learning. In it are described the effects of lesions involving the vertical, superior frontal and optic lobes. Boycott & Young (1950, 1955) have already shown these parts to be concerned in the establishment of memories of things seen, and have described their anatomy (Young, 1951; Boycott & Young, 1955). It will be shown here that the integrity of these parts also influences, but is not essential for, the establishment or retention of memories of situations experienced by touch.

MATERIAL

Octopus vulgaris Lamarck, of from 300–800 g., caught in the Bay of Naples, were used in these experiments. Each animal was kept separately as described by Boycott (1954) and fed on crabs or pieces of fish. Animals were weighed on arrival and operated upon after a quarantine period during which they were fed regularly and watched carefully for signs of damage (the results of rough treatment or prolonged exposure of parts of the skin to the air often only become apparent after several days). After use in one or more training experiments animals were killed and reweighed. The results of experiments with animals that ceased to feed regularly and lost weight were discarded. The brains of operated animals, fixed in neutral formol and stained using a modification of Cajal's method (Sereni & Young 1932), were sectioned to confirm the lesions. In the case of optic lobe removals, the optic lobes, which form discrete bodies joined to the brain only by the optic stalks, were examined under a dissecting microscope after removal and the completeness of the operation judged in this way. In all octopuses mentioned in this text as being without optic lobes the optic lobes were completely removed. Lesions to the vertical lobe were plotted as in Boycott & Young (1955). The diagrams on which lesions are plotted show the five gyri of the vertical lobe spread flat (in the living animal they are curved over the top of the brain) and a median longitudinal section through the supraoesophageal lobes. The nomenclature of the parts concerned is given in Fig. 3.

In the text individual animals are referred to by the number with which they were identified in our original protocols, e.g. B 10 or C 21. Numbers with the prefix B denote experiments made in 1955, and C, 1956. The suffixes B, NOL, NV, NSF denote, 'blind' (=optic nerves cut), 'no optic lobes', 'no vertical lobe', and 'no superior frontal lobe' respectively. Thus, for example, B 162 NVNOL signifies an animal having a lesion to the vertical lobe and without optic lobes; experiment made in 1955.

The temperature varied seasonally between 18 and 23° C., the maximum temperature variation during any one experiment being about 3° C. In winter the water circulating in the experimental tanks was heated.

OPERATIONS

Lesions were made to the brains of animals anaesthetized with 2–3% urethane in sea water. The central part of the brain was approached by means of a median longitudinal cut through the muscle layers and cartilage over the brain. After removal of the jelly surrounding the brain, a part of the latter was cut out and the wound closed by a stitch in the cartilage. The wound in the skin was left to close itself by contraction and in healthy animals stayed tightly contracted until it healed. Removal of the optic lobes was carried out through two lateral cuts over the eye-sockets; each optic lobe was set free by cutting the optic nerves and the optic tract peripheral to the optic gland, and was then removed leaving the peduncle lobe and other nervous structures associated with the optic stalk (Boycott & Young, 1956*a*) intact. Blinding by cutting the optic nerves was performed as described in Wells & Wells (1956).

EXPERIMENTAL

The ability of octopuses to learn to discriminate between members of a pair of test objects was investigated by training blind animals to take one object and reject the other. A pair of Perspex cylinders 2·5 cm. in diameter and 3 cm. long was used, one of these being smooth (P 4), and the other roughened by deep grooves 1 mm. in width cut into it either longitudinally at 3 mm. intervals (P 1), or circumferentially at 2 mm. intervals (P8). The objects are illustrated in Wells & Wells (1957); blind but otherwise unoperated animals can readily be trained to discriminate between either P 1 and P4 or P8 and P4. In the present series of experiments there were either 8 trials ('long-term' experiments) or 40 trials ('short-term' experiments) per day. At each trial one or other of the objects was presented by gently touching it against an arm of the Octopus under training; the animal was rewarded with a small crab or a piece of fish for accepting the 'positive' object, or given a 6–9 V. a.c. electric shock by means of electrodes fixed to the end of a probe if it accepted the 'negative' object. The movements made in accepting or rejecting objects have been described in detail elsewhere (Wells & Wells, 1956). Animals were operated several days before training was begun, and during the intervening period were fed regularly but given no experience of the test objects.

LONG-TERM EXPERIMENTS

In these experiments, there were 8 trials per day at intervals of not less than 1 hr., in a systematized order thus: first day, + − + − + + − −, second day, − + − + + − − +, and so on. There was a gap of about 14 hr. between the last trial on one day and the first on the next, and occasional longer intervals (usually every 7 days) when no trials were carried out for 36 hr. Except when stated (in some of the P8/P4 experiments) there was no attempt to select any particular arm when presenting the test objects to the octopuses.

Effects of removing the optic lobes (*NOL animals*)

Removal of the optic lobes produces considerable, though temporary, disturbances to the ability of *Octopus* to move about using its arms. For the first few hours after excision of the optic lobes, octopuses tend to sit on the floor of their tanks with their arms in confusion. If prodded they may grasp the probe, but do not move away as would a blind but otherwise unoperated animal. They do not appear capable of the co-ordinated movements of the arms necessary for normal walking. This inert phase is, however, temporary and after about a week they begin to move about and behave in all observed respects like animals blinded by cutting the optic nerves (for a full description of which see Wells & Wells, 1956). They will extend the arms towards a source of disturbance, such as a probe moving in the tank and move away when prodded, the arms now being well co-ordinated with one another. These octopuses succeed in climbing out of their tanks about as often as 'blind' animals, which have the optic nerves cut but the optic lobes remaining intact.

In Fig. 1, the performance of six animals without the optic lobes, trained to discriminate between P 1 and P4, is compared graphically with that of thirteen animals blinded by cutting the optic nerves. These results can also be expressed numerically in various ways, and it is perhaps easiest to compare the performance of animals in terms of an 'index of differential response' (Boycott & Young, 1957) that is independent of whether the animals err predominantly by acceptance of the negative object (as in the experiments shown in Fig. 1) or by rejection of the positive. The blind animals accepted the positive object on an average of forty-six and the negative on nineteen occasions during the experiments summarized in Fig. 1. The number of acceptances of the positive object minus the number of acceptances of the negative divided by the number of trials with each can be used as a measure of the degree to which the objects were separated and the figure so obtained will be referred to as the 'index of differential response' (I). Thus the index of differential response calculated for the whole period of training of the thirteen blind controls is $(46-19)/48=0{\cdot}56$, and for six animals lacking the optic lobes 0·63. These figures include the first week of training at the start of which the animals did not discriminate between the objects at all ($I=0{\cdot}0$); when this period is omitted from the calculation, the figures for blind and 'no optic lobe' animals are 0·70 and 0·84 respectively ($I=1{\cdot}0$ would indicate perfect performance).

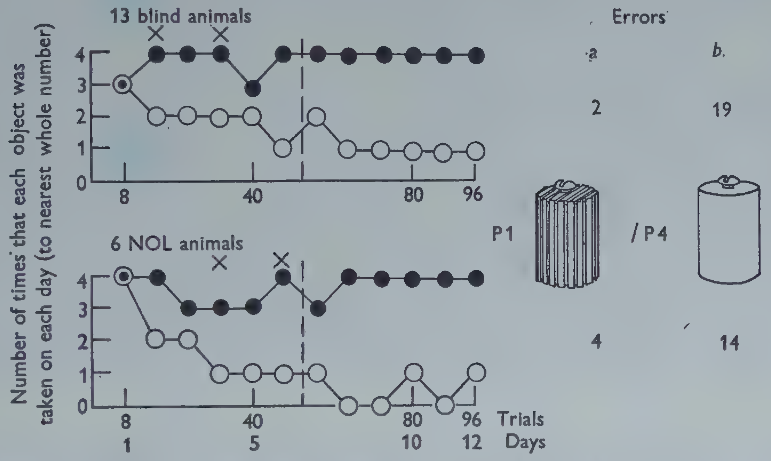

Fig. 1. Results of training to discriminate between the objects P 1 and P 4 (either as P 1+/P 4− or as P 4+/P 1−). The average performance of thirteen blind octopuses compared with that of six animals lacking the optic lobes. Eight trials per day (4+, 4−). ●, positive object; ○, negative object. A broken line indicates a break in training of 36 hr. $x-x$ indicates the period during which animals first averaged 75 % correct responses over 24 trials. Errors: a = number of errors due to rejecting the positive object, and b = number of errors due to accepting the negative object.

As can be seen from these figures and from Fig. 1, the performance of animals lacking the optic lobes was as good as, or better than, that of animals blinded by cutting the optic nerves. It is concluded that under these conditions, and at least in so far as discriminations of this order of difficulty are concerned, the optic lobes play no part in learning to make tactile discriminations.

Effects of lesions to the vertical lobe (NV animals)

Animals lacking parts or the whole of their vertical lobes were trained to discriminate between P 1 and P 4 under the same conditions. Their performance was always poorer than that of blind animals or of animals lacking the optic lobes. The results of training experiments with six animals having lesions to the vertical lobe and one animal with a lesion to the superior frontal lobe are given in Fig. 2 and Table 1. Most of these animals learned to discriminate between P 1 and P 4, but the proportion of errors that they made was always higher than that made by controls.

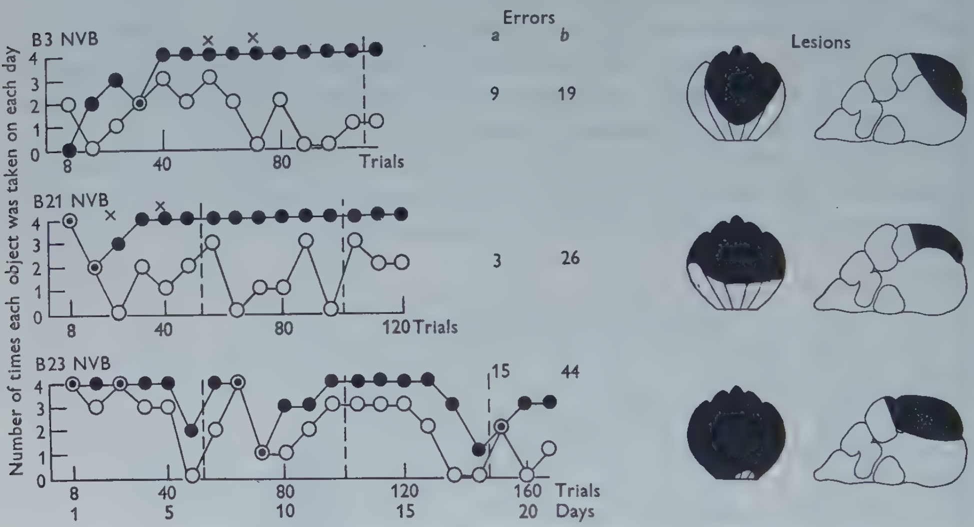

Fig. 2. Results of training three animals with lesions to the vertical lobe to discriminate between P 1 and P 4. Conditions of the experiments and plotting conventions as Fig. 1. Areas of nervous tissue removed are blacked out on a standard diagram which is explained in Fig. 3. B 3 NVB had 60 %, B 21 NVB had 65 % and B 23 NVB had 100 % of the vertical lobe removed (estimated to the nearest 5 %). The performance of five other animals with comparable lesions is given in Table 1.

Of the seven animals tested, three (B 39 NSFB, B 19 NVB and B 23 NVB), though subjected to prolonged training (88, 120 and 168 trials respectively, see Fig. 2 and Table 1), failed to reach a criterion (75 % correct responses over a period of 24 successive trials) that was reached by nineteen controls within an average of 37 trials; the remaining four animals averaged 62 trials to reach the same criterion.

Expressed in terms of the index of differential response described above, the performance of animals with lesions to the vertical lobe, considered over the whole period of their training, ranged from $I=0{\cdot}52$ (B 21 NVB) to $I=0{\cdot}05$ (B 19 NVB), with a mean for the six NV animals of 0·30 compared with an average of $I=0{\cdot}59$ for nineteen controls (blind animals plus animals lacking the optic lobes). The single animal having no superior frontal lobe (B 39 NSFB) did not discriminate between the objects at all ($I=0{\cdot}0$). (Removal of the superior frontal lobe can probably be regarded as functionally equivalent to complete removal of the vertical lobe since it deprives the latter of its afferent connexions; see Boycott & Young, 1955.) Comparable figures are obtained when periods excluding the first week of training are considered. Thus for the nineteen controls in the second week of training $I=0{\cdot}75$, and for the six NV animals $I=0{\cdot}36$.

Table 1. *Results of training three animals with lesions to the vertical lobe and one with a lesion to the superior frontal lobe to discriminate between the objects P1 and P4. Maps of the lesions are given in Fig. 3*

(Columns show the number of times that each animal took each object and total errors (column 'E') in each 8 trials (=1 day) of training. + = positive object. − = negative object.)

	Controls*						B15 NVB			B16 NVB			B19 NVB			B39 NSFB		
	1			2														
Amount of vertical lobe removed	—			—			60 %			60 %			90 %			100 %		
Total trials	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E
8	3	3	4	4	4	4	4	3	3	1	3	6	4	4	4	4	4	4
16	4	2	2†	4	2	2	4	2	2†	4	3	3	4	4	4	4	4	4
24	4	2	2	3	2	3	3	1	2	2	2	4	4	3	3	4	4	4
32	4	2	2†	3	1	2†	3	1	2†	4	3	3	4	3	3	4	4	4
40	3	2	3	3	1	2	3	1	2	1	3	6	4	4	4	4	4	4
48	4	1	1	4	1	1†	3	0	1	1	1	4	4	4	4	4	4	4
	One-day gap in training																	
	4	2	2	3	1	2	4	3	3	4	2	2	4	4	4	4	4	4
	4	1	1	4	0	0	2	2	4	0	0	4	4	4	4	4	4	4
	4	1	1	4	0	0	3	0	1	1	0	3	4	4	4	4	4	4
	4	1	1	4	1	1	3	3	4	2	0	2†	4	4	4	4	4	4
	4	1	1	4	0	0	4	4	4	2	0	2	4	4	4	4	4	4
96	4	1	1	4	1	1	4	4	4	4	2	2†	4	4	4	.	.	.
	One-day gap in training																	
	.	.	.	.	.	.	4	1	1	4	4	4	4	4	4	.	.	.
	.	.	.	.	.	.	4	1	1	4	4	4	4	4	4	.	.	.
	.	.	.	.	.	.	3	0	1	4	2	2	4	3	3	.	.	.
	.	.	.	.	.	.	3	1	2	4	4	4	.	.	.	.	.	.
	.	.	.	.	.	.	3	0	1	4	3	3	.	.	.	.	.	.
144	.	.	.	.	.	.	1	0	3	4	3	3	.	.	.	.	.	.
	One-day gap in training																	
	.	.	.	.	.	.	3	1	2	4	3	3	.	.	.	.	.	.
	.	.	.	.	.	.	3	2	3	4	4	4	.	.	.	.	.	.
168	.	.	.	.	.	.	4	1	1	4	2	2	.	.	.	.	.	.
Errors due to rejecting the +ve object	2/48			4/48			16/84			22/84			0/60			0/44		
Errors due to taking the −ve object	19/48			14/48			31/84			48/84			57/60			44/44		
Index of differential response‡	0·56			0·63			0·44			0·17			0·05			0·00		

* Average from (1) 13 blind octopuses and (2) 6 octopuses without optic lobes (from Fig. 1).

† Periods during which animals first made 75 % correct responses over a period of 24 successive trials.

‡ Calculated over the whole period of training.

When the performance of individual animals having vertical lobe lesions is considered (Figs. 2 and 3 and Table 1), it can be seen that, in general, those animals with the largest lesions made the greatest proportion of errors. Thus the indices for B23 NVB (100% of the vertical lobe removed—estimates are to the nearest 5%, in this case there was a small amount of vertical lobe tissue left, see Fig. 2) and for

B19 NVB (90% of the vertical lobe removed) were 0·30 and 0·05 respectively, while comparable figures for animals with smaller lesions such as B21 NVB (65% removed) and B3 NVB (60%) were 0·52 and 0·50 (*I* is calculated in each case over the whole training period; corresponding figures for the second week of training only are B23 NVB 0·25, B19 NVB 0·00; B21 NVB 0·66, B3 NVB 0·71). These figures suggest that the performance of animals in tactile training experiments is related to the amount of vertical lobe removed, in the same manner as Boycott & Young (1957) have demonstrated for visual discriminations.

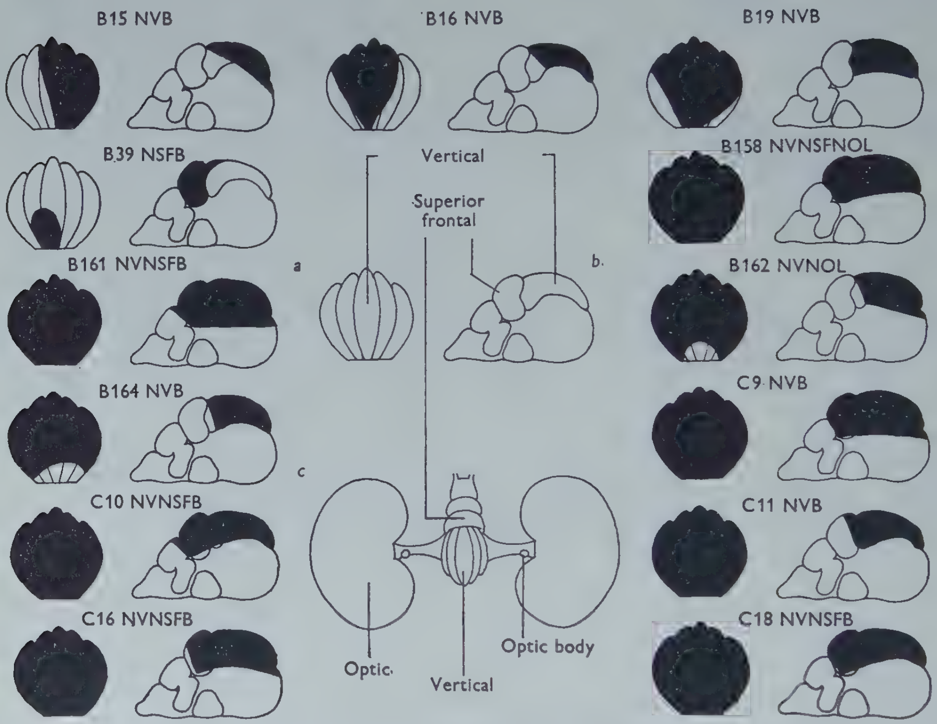

Fig. 3. Maps of lesions made to the brains of octopuses whose subsequent performances are given in Tables 1 and 2. In the centre of this figure the nomenclature of the parts concerned is shown on a set of standard diagrams (see page 379). Optic lobe removals were invariably complete, being made by section of the optic stalks between the lobes and the optic glands.

Experiments made under the same* conditions showed that octopuses could also be trained to discriminate between P8 and P4 (Table 2). When trained to make this discrimination, controls made a smaller proportion of errors than when required to discriminate between P1 and P4, a decrease that can be attributed to P8 having a greater proportion of groove to smooth surface than P1 (Wells & Wells, 1957).

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* Except that in three out of the seven experiments the test objects were always presented to the same arm of the Octopus under training as in the short-term experiments (see page 387). Table 2 shows that the selection of a particular arm did not affect the results of the experiments.

Table 2. *Results of training seven animals with lesions to the vertical lobe to discriminate between the objects P4 and P8. Four animals were trained using the second right arm only; three with no selection of arms*

	Controls			Trained using second right arm only												No selection of arms								
				C78 NVNSFB			C79 NVB			C82 NVNSFB			C83 NVB			C75 NVNSFB			C77 NVNSFB			C80 NVNSFB		
Amount of vertical lobe removed				100 %			95 %			100 %			100 %			100 %			100 %			100 %		
Total trials	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E
8	4	3	3†	2	1	3	3	1	2	3	4	5	4	4	4	4	4	4	4	4	4	4	4	4
16	4	2	2	3	2	3	0	1	5	4	4	4	4	4	4	4	4	4†	4	4	4†	4	4	4
24	4	1	1†	3	1	2†	0	0	4	4	4	4	4	4	4	4	1	1	4	1	1	4	4	4
32	4	1	1	1	0	3	3	0	1	4	3	3	4	4	4	4	1	1†	4	0	0†	4	4	4
40	4	1	1	3	0	1†	1	0	3	4	3	3	4	4	4	3	0	1	4	1	1	4	4	4
48	3	1	2	4	2	2	2	1	3†	4	4	4	4	4	4	4	2	2	4	1	1	4	4	4
										One-day gap in training														
	4	1	1	4	4	4	4	2	2	4	3	3	4	4	4	2	1	3	4	1	1	4	4	4
	4	1	1	4	1	1	4	0	0†	4	3	3	4	4	4	4	1	1	4	0	0	4	3	3
	4	0	0	4	0	0	4	1	1	4	4	4	4	2	2	4	0	0	4	1	1	4	2	2
	4	1	1	4	2	2	4	2	2	4	3	3	4	4	4	4	1	1	4	2	2	4	3	3†
	4	0	0	4	2	2	4	1	1	4	3	3	4	3	3	4	1	1	4	0	0	4	3	3
96	4	0	0	4	0	0	4	0	0	4	2	2	4	3	3	4	0	0	4	0	0	4	0	0†
										One-day gap in training														
	.	.	.	4	0	0	4	0	0	4	2	2	4	3	3	3	0	1	4	0	0	4	2	2
	.	.	.	4	0	0	4	0	0	4	4	4	4	4	4	4	1	1	4	0	0	4	3	3
	.	.	.	.	.	.	4	0	0	4	3	3	4	3	3	4	1	1	4	0	0	4	0	0
	.	.	.	.	.	.	.	.	.	4	2	2	4	2	2	.	.	.	4	0	0	4	3	3
	.	.	.	.	.	.	.	.	.	4	2	2	4	3	3	.	.	.	4	0	0	4	0	0
144	.	.	.	.	.	.	.	.	.	1	1	4	4	2	2	.	.	.	4	0	0	3	3	4
Errors due to rejecting the +ve object	1/48			8/56			15/60			4/72			0/72			4/60			0/72			1/72		
Errors due to taking the −ve object	12/48			15/56			9/60			54/72			59/72			18/60			15/72			50/72		
Index of differential response‡	0·73			0·59			0·57			0·19			0·15			0·63			0·79			0·30		

† Periods during which animals first made 75 % correct responses over a period of 24 successive trails.
‡ Calculated over the whole period of training.

In the present series of experiments eight controls reached a criterion of 75% correct responses in the first 24 trials of their training, compared with nineteen controls in the P1/P4 experiments which first attained a similar standard during trials 14–37 of their training, the indices of differential response for the two groups of controls over the whole period of their training (96 trials) being $I=0{\cdot}73$ (P8/P4) and $I=0{\cdot}59$ (P1/P4) respectively.

Similar comparisons can be made between the performance of animals with vertical lobe lesions. In the P8/P4 experiments all save one of the animals had lesions including total removal of the vertical lobe; in the P1/P4 experiments only three out of the seven animals (B19 NVB, B23 NVB and B39 NSF) had comparable lesions. The results of comparing the performances of animals with very large lesions from these two groups (*a*) with their respective controls, and (*b*) with each other, are given in Table 3. Table 3 shows that, like controls, animals with very large vertical lobe lesions made a considerably smaller proportion of errors in the P8/P4 experiments than in the P1/P4. It also shows how the performance of 'no vertical' animals, trained to discriminate between P1 and P4 and P8 and P4, approaches that of controls more closely in the case of the easier discrimination. Thus during the second week of training the indices for controls and 'no vertical' animals trained to discriminate between P1 and P4 were 0·75 and 0·08 respectively (difference 0·67); during the same period of training controls and 'no vertical' animals trained with P8 and P4 scored 0·88 and 0·54 (difference 0·34). Thus vertical lobe removal produced a relatively greater effect upon the performance of octopuses in the case of the more difficult discrimination. Similar results have been obtained with visual discriminations (Boycott & Young, 1957).

Table 3. *Mean indices of differential response during a sample period (second week of training; 49–96th trial)*

Discrimination	P1/P4	P8/P4
Controls	0·75	0·88
Animals with more than 90% of the vertical lobe removed	0·08	0·54
Difference in index between controls and 'no vertical' animals	0·67	0·34

Preliminary discussion of the results of the 'long-term' experiments

Training experiments with 8 trials per day (4+, 4−) at 1 hr. intervals show that:

(1) Removal of the optic lobes, comprising more than one-half of the total brain mass (Wirz, 1954), is without effect at least in so far as discriminations of the P1/P4 order of complexity are concerned.

(2) Extensive damage to the vertical lobe leads to an increase in the proportion of errors made by octopuses when attempt is made to train them to discriminate between P1 and P4 or between P8 and P4 under these conditions. This increase is

most marked in the case of the more difficult discrimination (P 1/P 4) and could be due to a number of causes, the most important possibilities being:

(*a*) Damage to the tactile integrative mechanisms that make it possible for the animal to separate (i.e. to distinguish between) the objects concerned.

(*b*) Damage to the mechanism allowing the animals to recognize the significance of situations once separated, i.e. to the mechanism permitting the occurrence of reward or punishment in combination with an object during one trial to affect the animals' reactions to the same object in subsequent trials.

(*c*) Damage causing deficiencies in the animals' ability to retain the effect of such
Possibilities (*a*) and (*b*) are eliminated by the experiments that follow, which
training between trials.
show that the effect of vertical lobe removal can be compensated, at least to a very considerable extent, by arranging trials at shorter intervals.

Sutherland (1957) has suggested a fourth manner in which vertical lobe removal might affect the performance of animals in discrimination experiments. In visual training experiments, animals from which the vertical lobe has been removed frequently make more errors than controls by attacking in an increased proportion of the 'negative' situations (Boycott & Young, 1955, 1956*b*), and he has suggested that one effect of the operation is to produce an over-all increase in the positiveness of the response. This hypothesis is not upheld by tactile training experiments in which the proportion of positive objects rejected by animals with vertical lobe lesions is comparable with, and often higher than, the proportion rejected by controls (Figs. 2, 3; Tables 1, 2 and 4). Young (1957) has recently shown that the proportion of attacks by untrained octopuses in visual situations is not increased by removal of their vertical lobes.

SHORT-TERM EXPERIMENTS

In these the training method was as before except that:

(1) The trials were at intervals of 5 min. instead of 1 hr. For convenience these were carried out in two groups of 20 trials on each day, the second group being carried out not less than 6 hr. after the beginning of the first. Each group was systematized thus: + − + − + + − − + − + − − + + − + − + −.

(2) The shocks and rewards were both rather smaller, 6 V. a.c. shocks were used instead of 9 V., and rewards (usually a whole or a half fish in the long-term experiments) were reduced to a size that the animals could be relied upon to finish eating in about 3 min.

(3) Wherever possible the test objects were presented to the same arm (usually the second right arm) of the animal under test throughout the period of the experiment. All short-term experiments were carried out in this way in order to make them comparable with others (to be reported in a later account) made with animals having brain lesions large enough to interfere with the transference of learning from one side to the other. The experiments with P 8 and P 4 (Table 2) have shown that selection of a particular arm under the long-term conditions does not significantly

influence the result in the case of animals having lesions similar to those quoted here. The differences in performance found under the long- and short-term conditions is, therefore, produced by altering the rate of training and cannot be attributed to arm selection.

Effects of lesions to the vertical lobe

In Table 4 are shown the results of training seventeen octopuses (of which eight, six blind and two without optic lobes, are controls) to make P 1/P 4 discriminations under the short-term experimental conditions.

All the animals, including some that had very large lesions including total removal of the superior frontal and vertical lobes (Fig. 3), learned to discriminate between these objects, each individual being trained until it reached a criterion of 75% correct responses (i.e. $I = 0{\cdot}50$) in a group of 20 successive trials. All thus attained a standard of accuracy of response that was reached only by controls and by those animals with the smallest lesions under the long-term conditions.

Table 4 shows that animals with lesions to the vertical lobes took rather more trials to learn to make the P 1/P 4 discrimination than did controls. Nine such animals (six of them entirely lacking the vertical lobe) reached a criterion of 75% correct responses in a group of 20 trials within an average of 3·9 such groups (78 trials) compared with eight controls that averaged 2·7 groups (54 trials).

REVERSAL OF LEARNED RESPONSES

1. *Under the short-term conditions*

The responses of animals that have learned to react positively towards one object and negatively towards another can be reversed if the animals are rewarded for accepting the previously negative object and punished for accepting the previously positive object. Reversal of responses was achieved in thirteen out of fourteen cases (seven animals with vertical lobe lesions and seven controls) in which animals were trained in this manner under the short term conditions, the single failure being a control that did not reach a criterion of 75% correct responses (reversed) within 280 trials. Details of these experiments are given in Table 4; in all cases there was an interval of 36 hr. between the end of the initial training and the start of reversed training.

At the start of reversed training both controls and animals with vertical lobe lesions typically made considerably more errors than would be expected from untrained animals, indicating a persistance of the effects of the initial training that was more marked in controls (I for the first 20 trials of reversed training $= -0{\cdot}36$) than in animals lacking the vertical lobe (I, over the same period $= -0{\cdot}26$). More rapid fading of the effects of the initial training probably accounts for the rather surprising result that under these conditions animals lacking the vertical lobes actually learned to reverse their responses in fewer trials than controls. In the experiments shown in Table 4, seven animals lacking the whole, or large parts, of their vertical lobes learned to reverse to a criterion of 75% correct (reversed) responses ($I = 0{\cdot}5$) in a group of 20 trials within an average of 3·7 such groups (74 trials) while six controls averaged 5·7 groups (134 trials).

Table 4. *Results of training animals under the 'short-term' conditions. There were 40 trials per day, in two groups of 20 (10+, 10−) trials*

Columns show the number of times that each animal took each object and total errors (column 'E') in each group of 20 trials. + = positive object; − = negative object.

	(a) Controls																							
	B165B			B166B			C2B			C3B			C4B			C5B			C6NOL			C19NOL		
Amount of vertical lobe removed	—			—			—			—			—			—			—			—		
Total trials	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E
20	10	7	7	10	7	7	10	4	4	2	3	11	6	7	11	8	0	2	10	6	6	10	8	8
40	8	6	8	10	8	8	.	.	.	4	2	8	2	0	8	.	.	.	10	4	4	10	9	9
60	10	4	4	9	3	4	.	.	.	7	3	6	5	2	7	.	.	.	.	.	.	8	4	6
80	9	3	4	10	4	4	Rev.			8	1	3	8	1	3	Rev.			Rev.			10	2	2
100	8	2	4	10	3	3	3	8	15	.	.	.	.	.	.	5	7	12	6	10	14	.	.	.
120	8	1	3	10	2	2	2	3	11	Rev.			Rev.			0	1	11	6	1	5	Rev.		
140	6	0	4	10	2	2	0	2	12	1	7	16	6	8	12	3	2	9	2	3	11	7	7	10
160	8	3	5	10	2	2	3	1	8	4	5	11	1	0	9	1	0	9	3	3	10	8	5	7
180	.	.	.	.	.	.	6	4	8	2	4	12	6	2	6	4	3	9	7	1	4	9	2	3
200	Rev.			.	.	.	8	2	4	3	1	8	0	0	10	0	0	10	10	4	4	9	1	2
220	0	6	16	.	.	.	10	4	4	1	1	10	5	1	6	3	1	8	10	2	2	.	.	.
240	0	6	16	.	.	.	8	2	4	1	1	10	4	2	8	0	0	10	10	1	1	.	.	.
260	0	3	13	.	.	.	8	1	3	0	2	12	8	2	4	0	1	11	9	2	3	.	.	.
280	6	2	6	.	.	.	9	0	1	3	3	10	7	4	7	2	1	9	.	.	.	.	.	.
300	3	1	8	.	.	.	10	1	1	8	1	3	6	1	5	1	1	10	.	.	.	.	.	.
320	4	2	8	.	.	.	10	0	0	7	1	4	9	1	2	6	3	7	.	.	.	.	.	.
340	9	4	5	.	.	.	.	.	.	8	0	2	0	0	10	1	0	9	.	.	.	.	.	.
360	8	5	6	.	.	.	.	.	.	.	.	.	8	2	4	5	2	7	.	.	.	.	.	.
380	9	2	3	.	.	.	.	.	.	.	.	.	6	0	4	.	.	.	.	.	.	.	.	.
400	6	2	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
420	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
440	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Errors due to rejection of + object*	13/80			1/80			0/10			19/40			19/40			2/10			0/20			2/40		
Errors due to acceptance of − object*	26/80			31/80			4/10			9/40			10/40			0/10			10/20			23/40		

* In initial training only.

Table 4 (*continued*)

	(*b*) Animals with vertical lobe lesions																										
	B15NVNSFNOL			B16NVNSFB			B162NVNOL			B164NVB			C9NVNSFB			C10NVNSFB			C11NVB			C16NVNSFB			C18NVNSFB		
Amount of vertical lobe removed	100 %			100 %			90 %			80 %			100 %			100 %			100 %			100 %			100 %		
Total trials	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E	+	−	E
20	5	10	15	9	10	11	10	10	10	10	7	7	7	5	8	10	5	5	1	2	11	7	8	11	8	3	5
40	4	7	13	10	7	7	10	10	10	10	9	9	10	7	7	.	.	.	1	1	10	9	6	7	.	.	.
60	10	7	7	10	9	9	10	8	8	10	10	10	10	1	1	.	.	.	4	1	7	8	4	6	.	.	.
80	8	5	7	10	7	7	10	7	7	10	9	9	.	.	.		Rev.		7	0	3	7	2	5		Rev.	
100	10	6	6	9	2	3	10	4	4	10	3	3	.	.	.	9	10	11	.	.	.	.	.	.	10	10	10
120	10	6	6	10	0	0	.	.	.	10	5	5		Rev.		10	5	5		Rev.			Rev.		9	6	7
140	10	1	1	7	2	5	.	.	.	10	3	3	4	10	16	10	5	5	3	4	11	2	4	12	10	10	10
160	10	1	1	6	1	5	.	.	.	10	5	5	5	6	11	10	2	2	2	2	10	2	6	14	7	7	10
180	.	.	.	.	.	.	.	.	.	.	.	.	10	6	6	.	.	.	5	5	10	2	2	10	10	9	9
200		Rev.			Rev.		.	.	.	.	.	.	10	5	5	.	.	.	4	2	8	2	1	9	10	8	8
220	4	10	16	1	3	12	.	.	.	.	.	.	10	6	6	.	.	.	9	3	4	9	2	3	10	6	6
240	9	10	11	3	4	11	.	.	.	.	.	.	10	2	2	.	.	.	6	0	4	6	2	6	9	4	5
260	10	10	10	10	5	5	.	.	.	.	.	.	10	5	5	.	.	.	5	1	6	10	4	4	10	5	5
280	10	10	10	9	1	2	.	.	.	.	.	.	10	3	3	.	.	.	9	2	3	8	3	5	10	2	2
300	10	10	10	10	3	3	.	.	.	.	.	.	.	.	.	.	.	.	10	0	0	7	4	7	10	5	5
320	10	10	10	10	4	4	.	.	.	.	.	.	.	.	.	.	.	.	10	3	3	8	1	3	10	3	3
340	10	10	10	10	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	0	6
360	10	8	6	9	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	10	0	0
380	10	6	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	3	4
400	10	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	2	3
420	10	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
440	10	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Errors due to rejection of + object*	13/80			9/80			0/50			0/80			3/30			0/10			27/40			9/40			2/10		
Errors due to acceptance of − object*	43/80			38/80			39/50			51/80			13/30			5/10			4/40			20/40			3/10		

* In initial training only.

2. *Under the long-term conditions*

Only two such experiments were made. Following the usual 36 hr. break, attempt was made to train B3 NVB and B21 NVB to reverse the learned responses to P1 and P4 shown in Fig. 2. These two animals were subjected to 168 and 144 trials respectively. During the first week of reversed training, I for B3 NVB was $-0{\cdot}29$ and that for B21 NVB $0{\cdot}0$; during the middle of the period of reversed training I was $0{\cdot}0$ for both, and in the last week $I=0{\cdot}04$ and $I=0{\cdot}13$ respectively. Thus the performance of both animals improved during the training period and there is reason to suppose that, had training been continued for long enough under these conditions, both would have eventually attained the standard of 75% correct responses over 24 trials reached by them in their initial training.

DISCUSSION

The results in this account were all obtained by post-operational training of animals from which the vertical and/or optic lobes had been partially or totally removed. The operated animals were required to distinguish between the members of a pair of objects of which they had had no previous experience; blind but otherwise unoperated animals can readily be taught to discriminate between these objects.

The performance of animals lacking the optic lobes was in all respects comparable to that of animals blinded by cutting the optic nerves only. It is concluded that the optic lobes play no part in learning to discriminate between objects by touch, and the term 'controls' used in this discussion refers to both blind and 'no optic lobe' animals.

Removal of large parts or of the whole of the vertical lobe markedly affects the performance of animals under the same training conditions. The number of errors made by such animals is always greater than by controls and can be related to the amount of the vertical lobe destroyed.

The difference between animals with vertical lobe lesions and controls was more marked when training was at a rate of 8 trials per day than at 40 trials per day. When trained to discriminate between the objects P1 and P4 at a rate of 8 trials per day, four animals with vertical lobe lesions averaged nearly 70% more trials than nineteen controls to reach a given criterion (75% correct responses in 24 successive trials) and three* failed altogether to reach this criterion although subjected to more than three times the number of trials required by controls. Trained to make the same discrimination until they reached a similar criterion (75% correct responses in 20 successive trials) at 40 trials per day, nine animals with comparable or greater lesions required only 45% more trials than their eight controls. None of the animals trained at this rate failed to reach the prescribed criterion.

* This includes an animal in which the superior frontal lobe was removed instead of the vertical. This operation deprives the vertical lobe of the greater part if not all of its afferent connexions (Boycott & Young, 1955) and can therefore probably be regarded as functionally equivalent to complete vertical lobe removal. Several of the animals that learned to discriminate when trained at a rate of 40 trials per day had lesions that included total removal of the superior frontal lobe.

These experiments show that the proportion of errors made by animals with vertical lobe lesions depends upon the rate of training, so that it is possible to compensate for vertical lobe removal to a considerable extent by conducting more trials per day at more frequent intervals. It can also be shown that removal of the vertical lobe has a relatively greater effect upon the performance of animals in difficult discriminations. Thus animals lacking 90% or more of the vertical lobe, trained at 8 trials per day, scarcely discriminated between P1 and P4, which controls distinguished with an accuracy of better than 75% ($I=0{\cdot}5$); other animals with comparable lesions trained at the same rate all learned to distinguish P8 from P4 with an accuracy approaching that of controls. It follows that, for any given problem and vertical lobe lesion, there must be a rate of training at which the trials are so far apart that the animal will fail altogether to show signs of learning; the critical length of this interval will decrease with increasing size of the lesion and increasing difficulty of the problem set.

The fact that it is possible to compensate for vertical lobe removal by arranging trials at closer intervals suggests that this part must be concerned in some way with the retention of the effects of training between trials. Retention presumably depends upon the persistence of a condition set up in the nervous system as a result of the sequence of events occurring during a trial, and the length of time that this condition remains capable of influencing motor responses to subsequent stimulation will depend upon its initial intensity and its rate of decay. We cannot tell, from the present series of experiments, whether the vertical lobe functions by increasing the intensity of the condition as it is set up (i.e. during the period in which the animal is receiving stimuli in connexion with the training situation) or by delaying its subsequent decay.

SUMMARY

1. Blind octopuses were trained to make tactile discriminations between the members of pairs of objects and their performance was compared with that of other blind animals having parts of the brain removed.

2. It was found that removal of the optic lobes, together constituting more than half of the total mass of the brain, did not affect the performance of animals in these discriminations.

3. Removal of the vertical lobe, on the other hand, produced deficiencies proportionate to the amount of tissue removed; these deficiencies were most marked in the case of the more difficult of the two discrimination problems used in the tests.

4. It was possible to compensate for loss of the vertical lobe to a considerable extent by arranging trials at more frequent intervals. Animals unable to learn a tactile discrimination when trained at a rate of 8 trials per day did so when trained at 40 trials per day.

5. When animals trained at the latter rate were subjected to reversed training, those lacking the vertical lobe re-learned in fewer trials than controls, indicating shorter persistence of the effects of pre-reversal training.

6. It is concluded that the vertical lobe is concerned with the persistence of conditions set up in the nervous system as a result of sensory experience. It is not known whether the vertical lobe serves to increase the initial intensity of these conditions, or to delay their decay.

The experiments reported in this paper were carried out at Naples at a time when M. J. W. was a member of the staff of the Stazione Zoologica di Napoli holding an Eli Lilly Fellowship. The work was prepared for publication in Cambridge during the tenure by M. J. W. of a Fellowship at Trinity College. The authors would like to thank Professor J. Z. Young, F.R.S., and Mr B. B. Boycott for helpful criticism during the work and for reading the manuscript.

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THE SPECTRAL SENSITIVITY OF THE ECHINOID *DIADEMA ANTILLARUM* PHILIPPI

By N. MILLOTT and M. YOSHIDA

Department of Zoology, Bedford College, University of London, Regent's Park, N.W. 1

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Several species of sea urchin show sensitivity to light, by the reactions of the whole animal or those of effectors such as the spines, podia, pedicellariae and chromatophores. The reactions have recently been reviewed by Millott (1954, 1956, 1957*a*).

The only accounts of the relative sensitivity of echinoids in various regions of the spectrum are those given by Millott & Yoshida (1956), who showed that the podia of *Psammechinus miliaris* are most responsive to shadows in the blue and green regions, and by Yoshida (1957), who showed that the chromatophores of *Diadema setosum* (Leske) respond, by pigment dispersion, most effectively in blue light.

Echinoids such as *Diadema* show spine movements, some of which are reflexes initiated by changes in light intensity, but the relative effectiveness of various regions of the spectrum has never been studied. Such studies are needed because they might provide some indication of the character of the photoreceptors and their pigment, about which very little is known. Since the reaction of the spines to shading is so constant and well marked, it is the obvious choice for an indicator reaction which may be used to determine the spectral sensitivity. A preliminary account has already been published (Millott & Yoshida, 1957).

METHODS

Isolated pieces of test were stimulated by shadows cast on to the internal or external surface. Sensitivity was determined on a basis of the minimum amount of light energy necessary to evoke responses to a standard shadow.

Apparatus

This is shown in Fig. 1. The piece of test, bearing spines and one radial nerve cord with its associated structures, was mounted in the tank *B*.

A light beam, produced by the lamp L_1 with a tungsten filament of known colour temperature calibrated by the manufacturers (Messrs B.T.H.), was interrupted by a spring-loaded shutter *X* to produce the stimulating shadow. The light passed through the lens system S_1, emerged as an almost parallel beam, the intensity of which was controlled by Kodak neutral density filters *F*. The voltage supplying the lamp was kept constant by means of a potentiometer and voltmeter. Spectral bands were obtained by interposing selected Balzer interference filters I.F., in the beam.

These passed wave-bands 8–11 mμ wide at 50% transmission, the spectral variation in transmission coefficient being shown more precisely in Fig. 2. The inside surface of the tank was enamelled dull black; the light beam entering by the window W_1 was disposed so that the beam centre was radial with respect to the curvature of the piece of test.

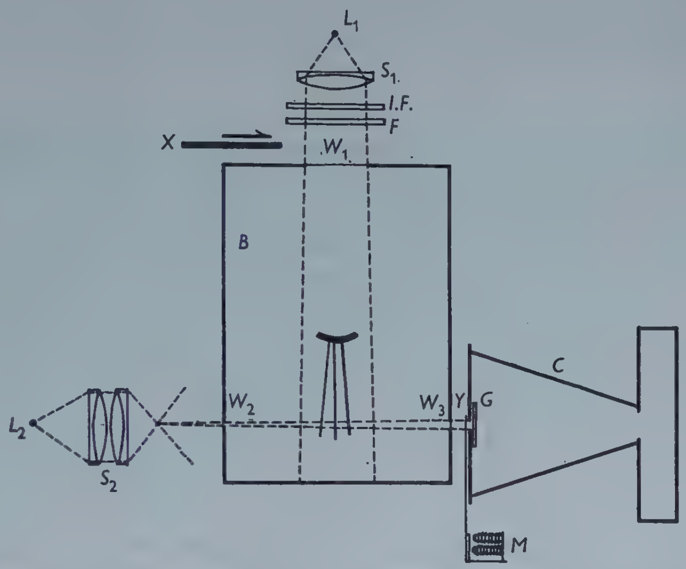

Fig. 1. Apparatus used to determine the spectral sensitivity of isolated pieces of test of *Diadema antillarum* (see this page). *B*, tank; *C*, camera; *F*,neutral density filter; *I.F.*, interference filter; *G*, ground-glass screen; L_1, L_2, light sources; *M*, time and stimulus signalling apparatus; S_1, S_2, lens systems; W_1, W_2, W_3, windows in opaque sides of tank; *X*, shutter; *Y*, slit.

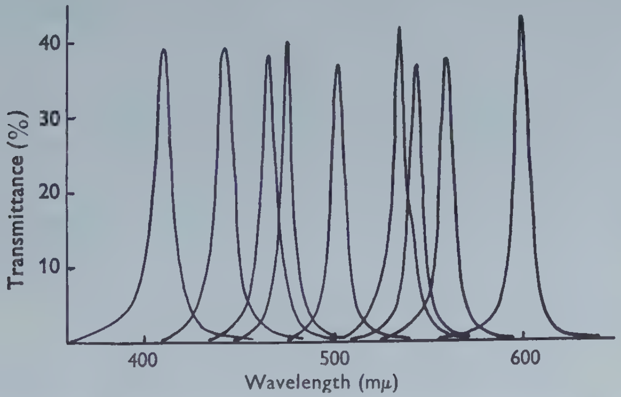

Fig. 2. Transmission coefficients of the Balzer interference filters used in experiments, determined spectrophotometrically.

The resulting spine movement was recorded on roll film carried in the oscilloscope camera *C*. A light beam from the lamp L_2 projected on to the camera through the slits W_2 and W_3, was focused by the lens system S_2 so as to produce a suitable sharp image of the spines at the slit Y on a ground-glass screen G located at the focal plane of the camera. The time at which the shutter closed was recorded automatically by the bar and solenoid M.

Experimental procedure

Experiments were performed in a dark room at 20–24° C.

The urchins used were brought from Madeira, and had been kept in aquaria for several months.

In the excised pieces of test the spines, other than those whose movements were to be recorded, were cut short. After being removed from the animal the pieces of test were allowed to recover and adapt themselves in aerated sea water in the dark room for at least 1 hr. before they were used. No piece was used for more than 4 hr.

The sea water in the tank B was aerated continually and the *maximum* variation in temperature of the water occurring in any series of experiments was 0·7° C.

In all cases the stimulus was given by closing the shutter after the piece of test had been illuminated by the coloured light for 3 min. To minimize mechanical shocks arising from the operation of the shutter and camera motor the piece of test was suspended by a clamp resting on a separate bench.

Since it was the effects of threshold stimuli that were under study, it was essential to record any spine movement, however small; the behaviour of the spines was therefore watched by two observers from different angles. The movements noted were compared with the photographic record in every case. Usually spines remained motionless unless stimulated. When spontaneous movements occurred no records were taken; the light was cut off and the experiment started again. If such movements persisted the preparation was discarded.

The sequence of experiments was considered carefully, the succession of filters used being varied in each series, after which checks were made by repeating some of the experiments.

Method of calculation

The relative amounts of light energy applied to the receptive surface of the urchin at various wavelengths were calculated from the energy distribution of the light source, the transmission coefficients of the interference and neutral density filters (measured on a 'Unicam' spectrophotometer S.P. 500), and the optical density of the sea water taken from data given by Harvey (1928). No account was taken of the lenses.

Thus the relative energy E_λ for each wavelength λ is given by

$$\log E_\lambda = \log H_\lambda - D_{I\lambda} - D_{S\lambda} - D_{T\lambda},$$

where H_λ is the energy radiated from the light source, regarded as a full radiator (obtained from Wien's formula); $D_{I\lambda}$, the optical density of the interference filters; $D_{T\lambda}$ that of the neutral filters; $D_{S\lambda}$ that of the sea water.

The relative energy over the spectral bands transmitted by each interference filter was calculated by adding the above values for wavelengths spaced at 2 mμ intervals.

RESULTS

Typical records are reproduced in Figs. 3A–C and show that the threshold is fairly sharp. Thus the interruption of a beam passed through filters, whose transmission may be represented by 3·7 log units, called forth the clear response shown in Fig. 3A; while the interruption of a beam, the intensity of which was less by only 0·2 log unit, was not followed by any response at all (Fig. 3C). A beam of intermediate intensity (differing from both by 0·1 log unit) produced a response which is clearly liminal (Fig. 3B). Repetition of the experiments from time to time in the way outlined on p. 396 showed that for each piece of test the threshold value was constant for at least 4 hr., which made it possible to obtain an action spectrum.

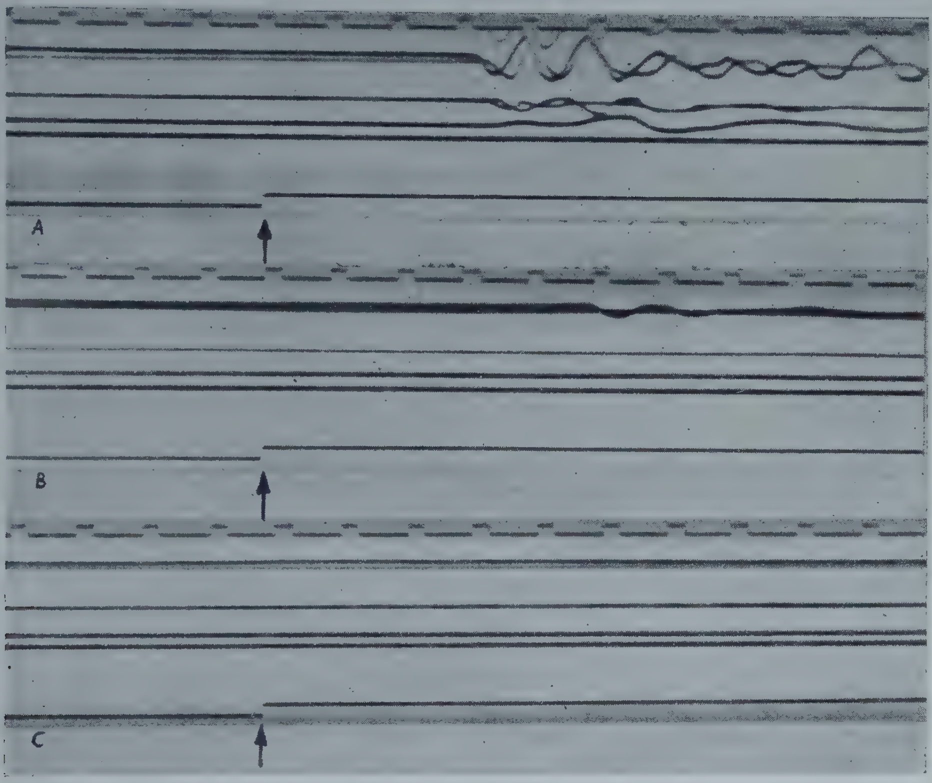

Fig. 3. Records of movements shown by spines borne on isolated pieces of test of *Diadema antillarum*, in response to shadow stimuli (see this page), in light intensities differing by 0·1 log unit. Time in seconds: arrows indicate interruption of light beam. A, brightest light; B, intermediate light; C, dimmest light.

The results of experiments in which pieces of test were stimulated from the inside and from the outside are summarized in Tables 1 and 2. In the left-hand column is shown, for each experiment, the relative amount of energy required at the point of maximum transmission of each filter to elicit a response to 100% shading, the lowest value being taken as unity. In the right-hand column is shown the relative sensitivity obtained from the reciprocals of these values, the highest being expressed as 100. These figures are therefore percentages of the maximum sensitivity.

When the light was shone on to the inside surface of the test, the sensitivity was consistently greatest at 465 mμ, falling off steeply on either side. The relatively constant sensitivity values enabled an average for the various experiments with each transmission band to be calculated (right-hand column of table 1) and a curve to be drawn (Fig. 4). The curve is only 70 mμ wide at 50% sensitivity.

Table 1. *Relative sensitivity at various wavelengths of isolated pieces of test illuminated from the inside. For explanation see text*

$\lambda_{max.}$	E1		E2		E3		E4		E5		Avera Sensi tivity (%)
	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	
410	4·00	25·0	—	—	—	—	3·85	26·0	—	—	25·5
442	1·78	56·2	2·50	40·0	—	—	2·30	43·5	2·05	48·8	47·1
465	1·00	100	1·00	100	1·00	100	1·00	100	1·00	100	100
475	1·21	82·6	1·30	76·9	1·21	82·6	1·28	78·1	1·03	97·1	83·5
501	1·52	65·8	2·00	50·0	1·67	60·0	1·43	69·9	1·56	64·1	62·0
534	3·49	28·7	3·52	28·4	—	—	3·57	28·0	—	—	28·4
544	6·12	16·4	—	—	—	—	—	—	—	—	16·4
558	10·2	9·80	15·2	6·58	—	—	10·7	9·35	—	—	8·5
598	79·4	1·26	171	0·58	—	—	72·7	1·38	—	—	1·0

Table 2. *Relative sensitivity at various wavelengths of isolated pieces of test illuminated from the outside. For explanation see text*

$\lambda_{max.}$	E6		E7		E8		E9		E10		E11	
	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)	Energy	Sensitivity (%)
410	4·09	24·5	—	—	6·83	14·6	—	—	—	—	3·72	26·9
442	1·44	69·4	1·36	73·5	1·51	66·2	—	—	4·40	22·7	1·00	100
465	1·00	100	1·00	100	1·00	100	1·63	61·4	1·31	76·3	1·23	81·3
475	1·03	97·1	1·01	99·0	1·25	80·0	1·00	100	1·00	100	1·06	94·3
501	1·55	64·5	—	—	1·66	60·2	1·85	54·1	1·85	54·1	2·05	48·8
534	5·68	17·6	—	—	4·35	23·0	5·84	17·1	8·47	11·8	1·82	55·0
558	—	—	—		20·7	4·83	—	—	17·5	5·71	—	—
598	—	—	—		208	0·48	—	—	—	—	—	—

The results of experiments in which the interrupted beam was shone on to the outside surface are summarized in Table 2 in which the data are similarly arranged. The results lack the uniformity of those obtained after inside illumination, for whilst experiments E6 to E8 indicate a maximal sensitivity at 465 mμ, E9 and E10 indicate a maximum at 475 mμ, and E11 shows several peak values, viz. at 442, 475 and 534 mμ.

This difference, though somewhat puzzling, does not necessarily indicate differing photoreceptor systems, for apart from the possible effects of interfering pigments, the results may be due to the inconsistency of the lighting conditions at the outside surface, the spines of which on altering their stance must necessarily cast varying shadows on it. In view of this uncontrollable factor little reliance can be placed on these results, though apart from those of E 11, they appear to indicate a maximal sensitivity somewhere between 465 and 475 mμ.

The results of stimulating the internal surface of the test in *Diadema* show clearly that, using the spine response as an index, sensitivity is greatest in the blue region of the spectrum.

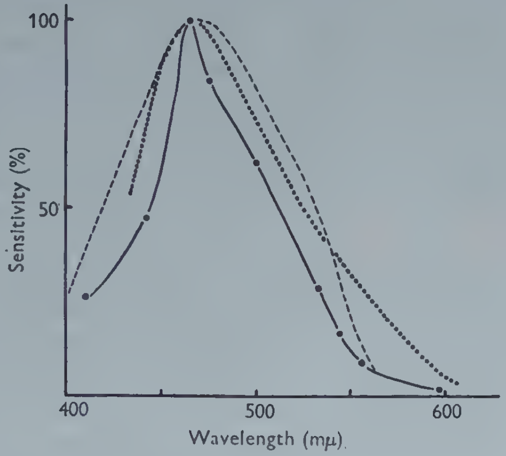

Fig. 4. Action spectrum of the spine response to shadows in *Diadema antillarum* (•—•—•), compared with that of the pigment dispersion in the chromatophores of *D. setosum*, (· · · · ·), and the optical density of naphthaquinone pigment in *D. antillarum* (- - - - - -).

DISCUSSION

In general, these results agree with those we have obtained previously. Thus the reactions of the podia of *Psammechinus* show that it is most sensitive to shading in roughly the same region of the spectrum. In the instance of *Psammechinus* the limitations of the methods used did not permit a more precise definition of the region of maximum sensitivity. The chromatophores of *Diadema setosum* are also most sensitive in the blue, with a maximum at 468 mμ (see Fig. 4 and Yoshida, 1957).

This degree of uniformity is noteworthy, not only because the responses of different effectors were used as indicators of sensitivity in each case, but also because the spine response is a nervous reflex (Millott, 1954), whereas the chromatophores behave as independent effectors (Yoshida, 1956). However, the receptive mechanisms for the spine and chromatophore responses differ; for though the maximum is about the same in each case, the action spectrum of the chromatophore response is considerably broader.

When the spectral sensitivity is compared with that of other animals, the curve of the spine response is remarkable, though not unique, in its maximum (465 mμ), which is situated so far into the blue region, and in its narrowness. The action spectrum for *Diadema* resembles some of those obtained from certain vertebrate eyes. Thus Granit (1942, 1943) and Dodt & Elenius (1956), studying the retinal responses of frog and rabbit respectively, obtained some action spectra showing maxima in the blue. The action spectrum obtained from the frog resembles that of the spine response of *Diadema*, not only in its maximum, but also in shape. It is yet too early to suggest a basis for such similarity.

A visual pigment with an absorption maximum in the same region of the spectrum as the maximum sensitivity of *Diadema* has been described by Dartnall (1952) in the tench (visual pigment 467), but its absorption curve is broader than the curve of the action spectrum of *Diadema*. Again, narrow band pigments, some having absorption maxima between 440 and 470 mμ, and one with a maximum at 465 mμ, have been produced by Ball, Collins, Morton & Stubbs (1948), by the action of strong acids on vitamin A or retinene$_1$, but their relation to naturally occurring visual pigments is still obscure.

In the instance of *Diadema* nothing whatever is known of the pigments responsible for photoreception. However, it is worth mentioning that naphthaquinone pigment, which when in the acid form shows an absorption maximum at 462/63 mμ, has been shown to be widely distributed in this urchin (Millott, 1957*b*). Such absorption at a point so near to the animal's maximal sensitivity is suggestive. This naphthaquinone is not known to be significantly photosensitive *in vitro*, although in this respect the pigment needs much more critical examination. It should be mentioned that such a property is not essential *a priori* in a visual pigment (see Dartnall, 1948), and there is some evidence that light-stable visual pigment complexes may exist (see Bliss, 1943). However, when the curve for the optical density of the pigment in ethanolic solution is compared with that of the action spectrum it is seen to be broader (Fig. 4). Thus apart from the near coincidence of the maxima, there is as yet no evidence to indicate that naphthaquinone is involved.

The action spectrum of the spine response makes it clear that *D. antillarum* is particularly sensitive to decreases in intensity of blue light. This finding is somewhat surprising, for although the existence of receptors responding in limited spectral regions can be envisaged as refinements in complex eyes, in the present state of knowledge it is difficult to appreciate the significance of such restricted sensitivity in an eyeless form. In the natural environment this will be translated into an increased awareness of shadows cast by intruders (including potential enemies) in such light. Blue light has been found to penetrate farthest into clear sea water (Utterbach, 1936; Vercelli, 1937), which means that if these urchins (which are common in shallow water) were to move into deeper water, provided it is clear, they will be moving into areas lighted so as to ensure they are maximally sensitive to shadows. Mortensen (1940) records that the species may occur at depths down to 400 m.

The precise importance of this as an ecological factor will not be clear until more is known of the vertical distribution of the species and of the optical properties of the sea water in which it is found.

SUMMARY

1. The spectral sensitivity of *Diadema antillarum* has been determined using as an index the spine response to shadows cast on the internal or external surface of the test.

2. With shadows cast on the internal surface sensitivity is consistently greatest at 465 mμ. On the external surface sensitivity is usually greatest at either 465 or 475 mμ.

3. The action spectrum is narrow and resembles some of those obtained from the frog's retina.

4. There is as yet insufficient evidence to indicate that the abundant naphthaquinone pigment ($\lambda_{\text{max.}}$ 463) occurring in this urchin, is involved in photoreception.

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REACTIONS OF SALMONID LARVAE TO LIGHT

BY P. M. J. WOODHEAD

Zoological Laboratory, University of Cambridge,
and Fisheries Laboratory, Lowestoft

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INTRODUCTION

The behaviour of fish larvae may be influenced strongly by light. The newly hatched larvae of several marine fishes have been described as 'photopositive', swimming persistently towards light of high intensities [e.g. *Gadus callarias* (Dannevig, 1932); *Clupea harengus* (Soleim, 1942; Buckmann, Harder & Hempel, 1953); *Centronotus gunnellus* and *Agonus cataphractus* (Franz, 1909); *Bathygobius soporator*, (Tavolga, 1950)]. On the other hand, the larvae of many freshwater fishes are 'photonegative', swimming away from light [(*Salvelinus fontinalis* (White, 1915); *Cyprinus carpio* (Wunder, 1935); *Richardsonius balteatus* (Weisel & Newman, 1951); *Salmo trutta* and *S. salar* (Stuart, 1953)]. Apart from depicting this behaviour in terms of an orientation, few attempts have been made to study more fully the reactions of fish larvae to light.

This paper describes the results of an investigation of the reactions to light of the brown trout larva (*Salmo trutta*), with further observations on the larvae of the rainbow trout (*S. irideus*) and the salmon (*S. salar*). It has been well established that salmonid larvae are strongly photonegative (Buckland, 1863; Hein, 1906; White, 1915; Gray, 1928*a*). Recently Stuart (1953) described brown trout larvae as unresponsive to light for the first 3 days after hatching; they then became photonegative until the final stages of yolk-sac absorption (just before feeding was about to begin), when their behaviour changed and they orientated towards the light. Stuart also observed this sequence of behaviour in the larvae of the salmon, and White (1915) has described similar changes in behaviour during the development of the American speckled trout (*Salvelinus fontinalis*).

Salmonid larvae were chosen for this study as a considerable body of data concerning their developmental physiology is available, and because they can readily be reared in the laboratory. It was hoped that the study of their reactions might suggest a general pattern of behaviour in relation to light in fish larvae and possibly might also be related to the later reactions of post-larval and adult fish.

MATERIAL AND METHODS

Brown trout (*S. trutta*) and rainbow trout (*S. irideus*) larvae were hatched from 'eyed ova' supplied by the Surrey Trout Farm, Nailsworth, Glos. The larvae used in the earlier (1954) experiments were hatched from ova in constant temperature tanks at 10° ± 0·1° C. in the Cambridge Zoological Laboratory, and were reared to feeding fry at this temperature. The rearing tanks were shielded from light by covering them with a cardboard box which excluded most of the daylight, but which was not entirely light-proof. Further larvae were obtained in 1955 and reared under similar conditions at the Fisheries Laboratory, Lowestoft. Mr Steven of the Nailsworth hatchery selected the eggs from single females in 1955 so that a homogeneous stock of trout larvae was available for experiments. Salmon larvae, hatched from eyed ova very kindly given by Mr H. Evans of the Gwynedd River Board, were reared under the same conditions as the trout. Larvae used in observations were kept separately in labelled 200 and 250 ml. beakers which were floated in the constant temperature tanks.

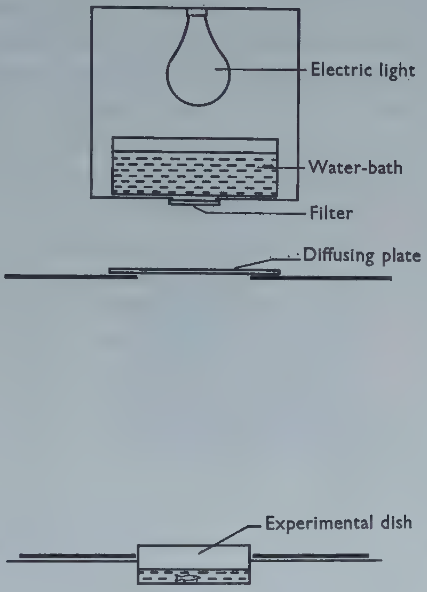

Fig. 1. The experimental apparatus.

The experimental apparatus was essentially a light source illuminating an experimental dish in which the behaviour of single larvae was observed. The light source consisted of a metal-box containing an electric light bulb and a glass dish filled with water to absorb radiant heat. Light issued through an aperture in the base of the box and immediately passed through a 5 cm. square Ilford, gelatin-film, neutral density filter. The light source was suspended 40 cm. above the experimental dish, and 8 cm. below the source was placed a plate of frosted glass to diffuse the light finally falling on the larva. Light intensities were measured at the water surface with a R.C.A. 931 A photomultiplier tube described elsewhere (Jones, 1955); the photomultiplier had a range from 10^3 to 10^{-3} metre-candles (m.c.); lower intensities were produced by using calibrated filters.

During the course of preliminary experiments it was noticed that the swimming movements of larvae were often arrested if they struck an obstacle with their heads. This reaction could cause serious interference with results but was avoided by the use of a circular experimental dish in which larvae usually swam round the perimeter, not encountering corners. The experimental dish was a glass crystallizing dish of 12 cm. diameter filled with water to a depth of about 3 cm. The basin was painted white on the outside, this white coat was later covered with matt black paint, so that it appeared white when viewed from the inside, but was black outside to reduce stray light reflexion. Matt black light screens were placed around the dish and the frosted glass diffusing plate, as shown in Fig. 1, and vertical screens were also placed around the whole apparatus to prevent the reflexion of stray light from the walls of the observation room.

The 1954 experiments were carried out in a dark room separate from the temperature-controlled rearing tanks, and it was not possible to observe the larvae at a temperature of 10° C. By placing the experimental dish inside a water-bath through which a slow water circulation was flowing, the temperature was kept between 12° and 14° C. during experiments. During this series the experimental larvae were kept in the dark at about 13° C. for at least 1 hr. before observation in order to become adapted to the temperature change. The 1955 experiments were all carried out at 10° C. so that the larvae were always at the same temperature.

The larvae were not continuously active in the experimental dish, and a measure of their activity could be obtained by simply observing the total time for which they were active during a given observation period; this was recorded either directly with a stop-watch, or else marked on a smoked kymograph drum by using hand-operated morse-keys connected to relay markers. As the larvae were found to be very sensitive to vibration and tactile stimulation the drum-recorder and driving motor were mounted on a separate bench.

The procedure used during experiments was to place a dark-adapted larva in the experimental dish and leave it for 20 min. in total darkness. The light was then switched on and observations made for 15 min.; the larva was then replaced by another larva, or else left for a period of 20–30 min. in total darkness before commencing a further 15 min. period of observation. When more than two observations were being made on one larva, it was removed from the experimental dish for 1 hr. between successive observations. In experiments carried out at low light intensities it was essential for the observer to be completely dark-adapted in order to see the larvae.

RESULTS

Experiments with brown trout (Salmo trutta)

Brown trout larvae were not continuously active in the experimental dish, but lay on the sides of their yolk-sacs for long periods, their only movements being those of respiration and a rapid and persistent movement of the well-developed pectoral fins, probably also of respiratory function. Periodically the larvae became active, their activity was characterized by vigorous, although inefficient, swimming movements. These movements might propel the larvae round the dish or might be of insufficient strength to produce forward progression, although they would bring larvae off their sides to lie above the yolk-sac.

A period of activity generally began with a number of gentle flexions of the body which were then replaced by more vigorous movements producing forward locomotion. After such a spell of activity the larva rolled slowly over on its large yolk-sac to lie on its side once again. In many cases this rolling movement appeared to stimulate the larva to a further burst of activity and this pattern might be repeated several times. In such cases the duration of the bursts and also the strength of the swimming movements gradually decreased until the larva finally came to rest. This has been shown for two trout larvae in Fig. 2 by taking smoked-drum recordings of single periods of activity and from these recordings estimating the percentage activity observed in 3 sec. intervals throughout this period. This sequence of an initial burst of fairly intense activity gradually decreasing with time was not the invariable pattern of the response but was the most typical form.

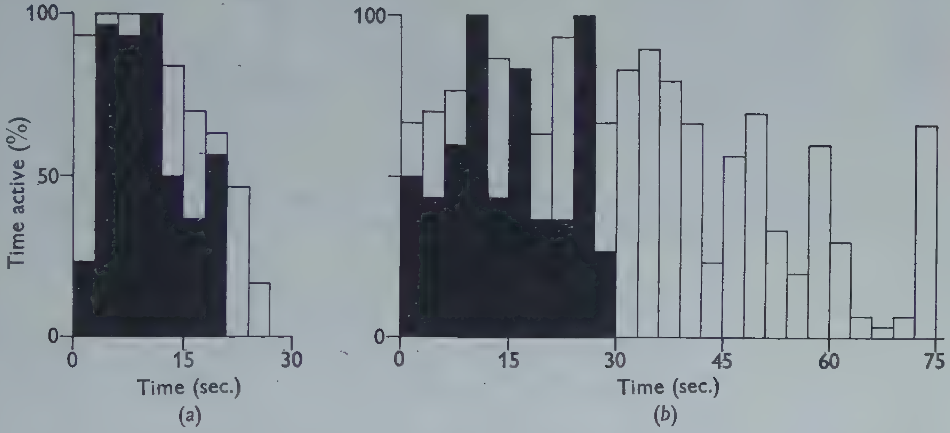

Fig. 2. Time-course of a single burst of swimming in two trout larvae (*a*) and (*b*), showing the gradual decrement in activity. ■, swimming movements propelling the larva forward; □, swimming movements of insufficient strength to propel the larva forward.

Photo-orthokinesis

The brown trout larvae exhibited a high photo-orthokinesis (Fraenkel & Gunn, 1940), the time for which individual larvae were active during an observation period increasing in direct proportion to the logarithm of the light intensity, over a limited range (Fig. 3). Larvae were found to be sensitive to light on the day of hatching and an orthokinesis was demonstrated in such larvae, although they were only active for about 1% of the total observation time and their swimming movements were generally of insufficient strength to propel them forward.

At very low light intensities the larvae were found to have a low level of activity which did not increase with increasing light intensity until a certain light level was passed; it was considered that this intensity was the light sensitive threshold for the kinesis (Fig. 3). The threshold was generally about 0·005 m.c.; it was as high as 0·05 m.c. for many of the younger larvae, but tended to become lower during development, lying in the region of 0·0005 m.c. for some of the later stage larvae.

Above 0·05 m.c. the relationship between light intensity and activity approximated to a straight line, especially in the later stages of development. An upper limit for the kinesis was demonstrated in some older larvae, and although this varied individually, it generally lay between 10 and 100 m.c. The upper limit was not determined for most of the younger larvae but seemed to be higher than 100 m.c.

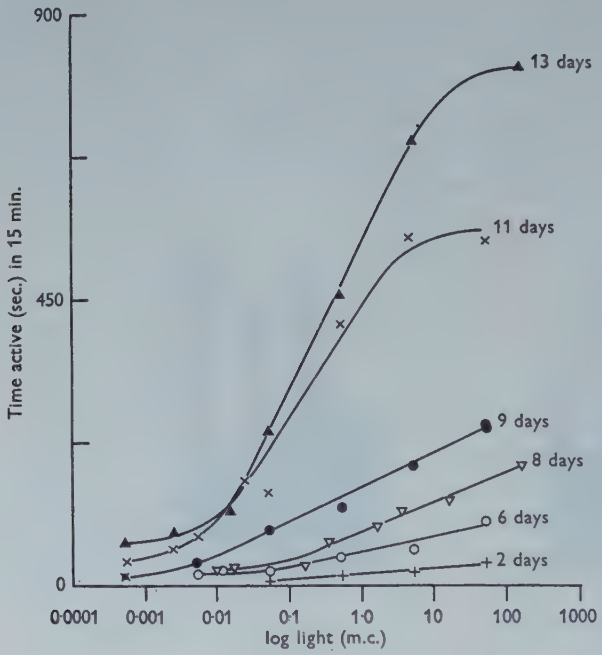

Fig. 3. Time spent moving by trout larvae at different light intensities. Results for single larvae of different ages.

The time-course of the response

Discussing the role of kineses in animal behaviour, Fraenkel & Gunn (1940) point out that in the case of a klino-kinesis fairly rapid adaptation of the response will increase the rate of aggregation of organisms. On the other hand, in an orthokinesis, adaptation is not an essential part of the mechanism of aggregation; indeed the mechanism will be less efficient if adaptation occurs. In order to determine the course of the response of trout larvae with time some observations were carried out for longer periods than the usual 15 min.

The results for three typical larvae illuminated at 5 m.c. are shown in Fig. 4; in the figure the total observation period has been split arbitrarily into 3 min. subdivisions, and the activity occurring in each subdivision has been plotted against time. Although the over-all level of activity of individual larvae varied considerably, larvae generally showed a rhythm of activity. Their activity slowly built up to peaks at roughly 15 min. intervals and after each peak activity fell once again to a low value. It seems possible that processes of fatigue and recovery may have contributed to the formation of this rhythm. No evidence of adaptation to the light stimulus was apparent in larvae of 8–10 days old after 90 min. illumination.

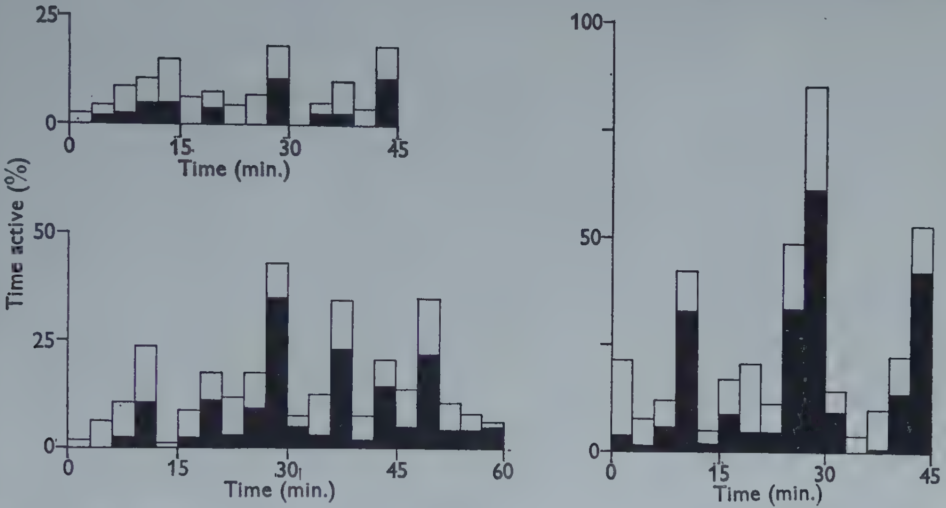

Fig. 4. The activity of three typical trout larvae (10 days after hatching at 10° C.), plotted in 3 min. intervals, over prolonged periods of illumination at 5 m.c. ■, swimming movements propelling the larva forward; □, swimming movements of insufficient strength to propel the larva forward.

Changes in activity with development

At an early stage in the experiments it became apparent that the general level of activity increased as larvae grew older; this is well shown in Fig. 3. An index of the change in the magnitude of the orthokinesis with development was obtained by studying the activity of about twenty individuals at two light intensities (50 and 0·005 m.c.) at intervals during their development. The mean values for the activity of larvae at these intensities on successive days after hatching are shown in Fig. 5 and Table 1.

At 50 m.c. the general level of activity increased slowly over the first 6 days, and then rose more rapidly until finally, about 15 days after hatching, the larvae were active throughout the observation period. At 0·005 m.c. the increase in activity was initially slower, but in the later stages rose very rapidly to reach full activity at almost the same time as at the higher light intensity.

It should be emphasized that the mean values shown in Fig. 5 were obtained with a mixed batch of larvae which had not hatched on the same day. The age of each was taken from its day of hatching, and, on completion of the experiment, it was found that, because larvae of different ages (with respect to their day of hatching) had been used on each day of observation, each mean was not derived from a similar number of observations. This is shown in Table 1 where it can be seen that there is considerable variation in the number of larvae of different ages which had been examined in this experiment. Nevertheless, the results show clearly the course of the changes in activity at two different light levels. All other results reported here were obtained with homogeneous batches of larvae which had all hatched on the same day.

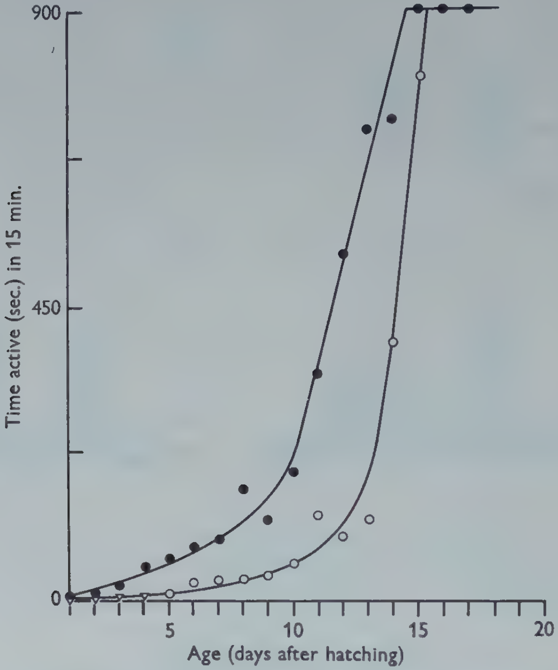

Fig. 5. The increase in activity, at two light intensities during the course of development of the brown trout larva (1954 experiments). ●, activity at 50 m.c.; ○, activity at 0·005 m.c.; ▽, activity at 0·05 m.c.

Table 1

(Numbers of larvae used indicated in parentheses.)

Days after hatching	Time active (sec.) per 15 min.			
	50 m.c.	S.D.	0·005 m.c.	S.D.
1	10·5 (19)	7	4·5 (1)*	—
2	10·5 (12)	7	3·5 (4)*	3
3	21 (13)	14	4 (3)*	4
4	50·5 (12)	36	7 (1)*	—
5	67 (12)	60	13 (11)	12
6	83 (6)	66	28·5 (4)	23
7	93 (6)	66	31·5 (3)	13
8	171 (12)	122	31 (12)	22
9	122·5 (3)	23	37·5 (4)	27
10	197 (2)	—	55 (2)	—
11	358 (8)	169	132·5 (7)	60
12	531·5 (5)	144	98·5 (6)	85
13	718 (2)	—	121·5 (4)	56
14	736·5 (11)	218	395 (12)	308
15	900 (8)	—	798·5 (8)	219
16	900 (9)	—	896 (11)	16

* Larvae examined at 0·05 m.c.

During the later stages of development the larva tended to leave the bottom more often when swimming, and its swimming movements appeared more efficient. This may, in part, have been due to the increase in size of the embryo and decrease in size of the yolk-sac; but there also appeared to be some change in the nature of the movements themselves, the vigorous lateral lashing of the tail observed in younger larvae was increasingly replaced by the slower caudal conduction of undulatory waves, characteristic of juvenile trout fry.

It was at this point, where full activity was exhibited at all intensities, that the behaviour of the larvae changed. Whereas larvae had previously exhibited a strong negative phototaxis, young fish swam freely in mid-water with many changes in direction but apparently heedless of the light. Sprenger's (1945) observation that the persistent pectoral fin movements disappeared at this stage was confirmed, the fins beginning to move in co-ordination with general body movements as in adult fish. The young fish snapped at any small object floating in mid-water or at the water surface and feeding soon began. Small air bubbles appeared peculiarly attractive to the larvae which possibly filled their air bladders in this way as has been suggested by Ledebur (1928); certainly the young fish now appeared to have less tendency to sink and maintained their positions in mid-water with fairly slow and easy movements compared with the very vigorous activity previously required to take them off the bottom; Grove & Newell (1939) have also described changes in the specific gravity of trout larvae after hatching. Fish in this early feeding or pre-feeding stage did periodically sink to the bottom to lie inactive for a considerable time, but active periods appeared to last for hours instead of seconds or minutes as had been the case in the larval photokinesis.

Experiments with rainbow trout (Salmo irideus) *and salmon* (Salmo salar)

The experiments carried out on brown trout were extended to the larvae of rainbow trout and salmon. A photo-orthokinesis was also demonstrated in both of these larvae. The rainbow trout larva was smaller than the brown trout larva on hatching and appeared less well developed. Rainbow trout were even less active than the brown trout after hatching and in the earliest stages a number of the larvae still exhibited incomplete swimming movements, occasionally contracting one side of the body only, to form a 'C-shape', or over-contracting in a flexion, the tail being brought right round crossing the body behind the head to form a 'figure-8'; this might be repeated once or twice to each side. Leghissa (1942) has described similar movements in salmonid larvae prematurely excised from their eggs, and he has related them to incomplete development of the central nervous system.

In 1954 a preliminary investigation of the change in activity with development in the rainbow trout revealed that, with respect to the time of hatching, the rainbow trout took about a week longer than the brown trout to reach the point of full activity at all light intensities. The results were confirmed in 1955 with brown trout and rainbow trout larvae which had hatched on the same day from homogeneous batches of eggs spawned by single females. It was found that brown trout larvae

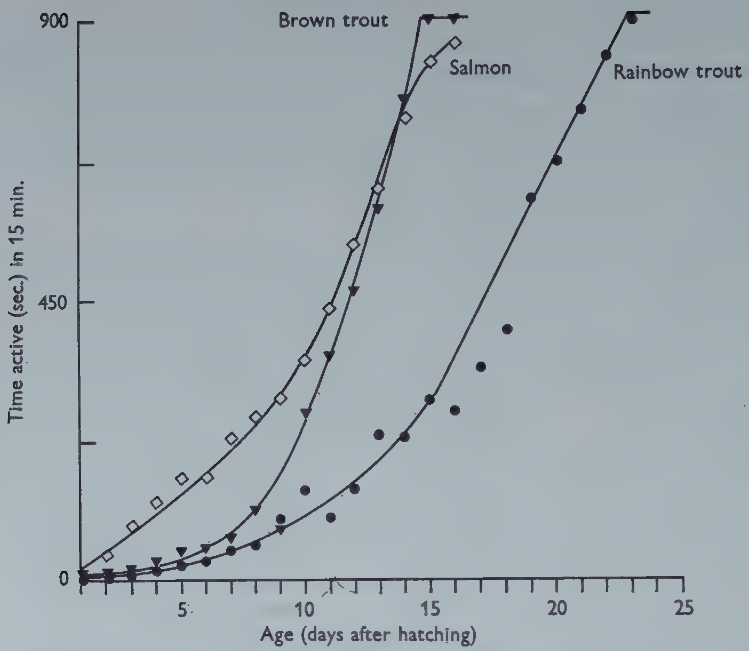

Fig. 6. The increase in activity at 4·6 m.c. of the brown trout, rainbow trout, and salmon larvae during the course of development (1955 experiments). ▼, brown trout; ●, rainbow trout; ◇, salmon.

Table 2

(Numbers of larvae used indicated in parentheses.)

Days after hatching	*S. trutta*		*S. irideus*		*S. salar*	
	Time active (sec.) per 15 min.					
	Result	S.D.	Result	S.D.	Result	S.D.
1	No. obs.		2 (8)	1	12 (8)	8
2	10 (7)	7	6 (8)	6	42 (8)	14
3	21 (8)	9	10 (8)	10	91 (10)	38
4	35·5 (8)	20	18·5 (8)	5	126 (10)	46
5	50·5 (8)	21	28 (8)	11	159·5 (10)	66
6	52·5 (7)	26	29·5 (8)	9	168 (12)	68
7	67·5 (11)	21	51 (8)	26	230·5 (12)	82
8	115 (11)	62	56 (8)	18	258 (12)	96
9	84·5 (8)	49	100 (8)	68	293 (11)	75
10	259 (12)	134	148 (7)	53	353 (11)	84
11	363 (12)	113	101 (8)	69	438 (11)	90
12	462 (12)	187	148 (9)	100	538 (10)	91
13	591 (12)	153	235 (8)	82	624 (10)	68
14	768 (12)	191	229·5 (10)	127	738·5 (10)	86
15	900 (12)	—	291 (7)	167	830·5 (11)	114
16	900 (12)	—	270·5 (7)	117	861·5 (11)	63
17	—	—	339·5 (9)	143	—	—
18	—	—	397·5 (8)	119	—	—
19	—	—	610·5 (7)	106	—	—
20	—	—	669 (8)	94	—	—
21	—	—	747 (9)	105	—	—
22	—	—	835 (9)	73	—	—
23	—	—	887 (9)	27	—	—

reached full activity 15 days after hatching, whereas rainbow trout larvae took 23 days to reach this point (Fig. 6 and Table 2).

An attempt was also made to compare the development of activity in the salmon larva with that in the trout. The larvae of the salmon were larger and more active on hatching than those of either the rainbow or the brown trout. The curve of change in activity with development rose steeply at first, but in the later stages this rapid rise was not continued as had been expected (Fig. 6). Furthermore, the later salmon larvae often arched their bodies laterally and exhibited a faint quivering movement. This behaviour was not observed in the trout and did not seem to correspond to the type of activity described previously, but, as the larvae were not lying totally inactive, this quivering movement was recorded as 'activity' for purposes of comparison.

It seems probable that the curve of change in activity for the salmon larva is not strictly comparable with those for the two species of trout. Comparing the post-larval behaviour of feeding salmon and trout fry, Stuart (1953) observed that trout fry swim actively, maintaining station in mid-water, whereas salmon fry remain in groups resting on the bottom only rising to seize food and then returning to the bottom. Thus it seems that throughout development trout larvae are approaching a condition of persistent swimming for long periods, and activity probably gives a reasonable index of the rate of development. In the salmon, on the other hand, although the kinesis is present in the larva, the fry is not persistently active. It may be that this difference in post-larval behaviour is connected with the quivering activity observed in salmon larvae, and in particular, with the reason why many salmon larvae never achieved full activity during later observation periods.

DISCUSSION

Although it is well established that trout larvae are negatively phototactic, a true photo-orthokinesis (Fraenkel & Gunn, 1940) does not appear to have been described for these animals. White (1915) claimed that rainbow trout (*S. irideus*) and American speckled trout (*Salvelinus fontinalis*) larvae were photokinetic, because when she drew back the curtain of the darkened room in which they were kept, they 'became active for a minute or two', after which their activity ceased. No relationship between the reaction and the stimulus strength was demonstrated, and White assumed that the reaction was of short duration only; this does not describe an orthokinesis, as defined by Fraenkel & Gunn (1940): 'Speed or frequency of locomotion dependent on intensity of stimulation. Undirected reaction: no orientation of axis of body in relation to the stimulus; locomotion random in direction'. White's work was carried out before this terminology was introduced and she did not realize the true nature of this reaction. It is of interest that a photo-orthokinesis is probably also exhibited by the larvae of the Pacific salmon, *Onchorhyncidae*, as Smith (1916) found that the larvae of the chinook (*O. tschawytscha*) and humpback salmon (*O. gorbuscha*) were very active in the light but lay almost inactive in the dark, like the larvae of the salmonids reported here.

In their normal environment trout larvae hatch from eggs laid in the redd in a gravel bed. They remain within the portection of the bed, living on the contents of their yolk-sacs until these are half to two-thirds absorbed, when they leave the gravel and begin to feed.

A photo-orthokinesis, coupled with a negative phototaxis, would appear to fit the larva well for its environment. It will lie relatively inactive in the darkness within the gravel bed. If exposed it will become active, its activity being proportional to the light intensity. If this activity were unorientated it would tend to take the alevin out of the light (and probably back into the bed), but the taxis would add an orientating factor making the return to cover, out of the light, more rapid and efficient. This behaviour is well described by Roule (1932) and Stuart (1953).

The sequence of changes in behaviour reported for salmonid larvae by Stuart (1953)—initial insensibility to light, followed by photonegative behaviour, the larvae finally abandoning this photonegative behaviour when about to start feeding—is confirmed in the main by the present investigation. However, larvae were not insensitive to light during the first few days after hatching, but did respond to illumination, although at a very low level of activity. Reporting on the behaviour of brown trout larvae in illuminated tanks, Stuart (1953) states that: 'The ova hatched and the alevins with large globular yolk-sacs lay on their sides at the bottom of the tank where they remained without altering their position for about 72 hr. (at about 5° C.) with alternating periods of light (from one direction) and complete darkness. At the end of the 72 hr. period, however, there was an almost simultaneous migration to the end of the tank furthest from the source of the light....Activity in the alevins was very marked with their bodies flexing in rapid swimming movements although forward progression was halted by the end wall of the tank. Throughout the following weeks this form of activity decreased as the yolk sac became smaller, but the orientation away from the source of the light was constant'.

This description is not confirmed by the results reported here as no evidence of a sudden change in activity was observed at this stage of development in any of the salmonid larvae examined, and, unless the kinesis adapts after several hours or days in the light, it is also difficult to account for Stuart's observation of decreasing activity with development. In this respect the observations of Dean (1896) on the larval behaviour of the holostean, *Amia calva* are of interest. Although Dean did not indicate any orientation with respect to light (unless 'a tendency to swim head downwards' in an illuminated tank indicates a negative phototaxis) he observed an increasing degree of activity with development and a change in swimming behaviour some 2 weeks after hatching.

It has often been assumed that trout larvae cease to exhibit photonegative behaviour, and leave the gravel bed to begin feeding, just as the yolk-sac is going through the final stages of resorption—'just before the last vestiges (of yolk) disappeared the alevins, which had hitherto sought the darkest corner of the tank, reorientated themselves...in a direction facing the light' (Stuart 1953). This is not borne out by the present results. Gray (1928*a*) found that the yolk-sac and contents constituted about 50% of the total wet weight (70% of the dry weight) of the larva on the sixtieth day after fertilization (i.e. the fifteenth day after hatching when the larva reaches full activity and its behaviour changes) in brown trout larvae reared at 10° C. It seems very reasonable that the larva should leave the protection of the bed and begin feeding whilst the yolk-sac still contains a considerable reserve of food material. This material can be drawn on in the event of the young fish not finding suitable food organisms immediately available. In this respect the brown trout larva appears better equipped than the rainbow trout since the yolk-sac and contents only constitute 22% of the total wet weight of the latter (Smith, 1947) at the time when the change in behaviour occurs.

Comparing the development of the rainbow and brown trout larvae, it would at first appear that the rainbow trout develops most rapidly since it hatches about 9 days earlier than the brown trout at 10° C. (Gray, 1928*b*; Smith, 1947). However, if the times at which full activity was achieved by the larvae in these experiments, are compared, it will be seen that this point is reached at about the same time after fertilization in both species (Table 3). Gray (1928*b*) has shown that in the brown trout the hatching mechanism is to a limited extent independent of the degree of development of the larva, and it is suggested that the two species may develop at much the same rate but that there is a differential behaviour of their hatching mechanisms (i.e. time of activation of the hatching-protease); from this point of view the actual time of hatching may be considered not to be very critical in trout. Of course, this is certainly not the only difference between the development of the two species. For reasons discussed above, the later behaviour of the salmon larva does not seem to be strictly comparable with that of the trout larva and so no attempt has been made to fit the salmon into this scheme.

Table 3

	S. trutta	*S. irideus*
Days from fertilization to hatching at 10° C.	42–45 (Gray, 1928*b*)	34–36 (Smith, 1952)
Days from hatching to full activity at 10° C.	15	23
Total number of days	57–60	57–59

In 1898 Whitman wrote that 'Instincts and organs are to be studied from the common viewpoint of phyletic descent', and more recent work has confirmed that instinctive behaviour may be analysed with the precision which has been applied in morphological studies. The increasing degree of activity shown by developing trout larvae may be considered as part of the general growth pattern, to which it bears a definite relationship. This is shown in Fig. 7 in which the increase in size (wet weight) of the embryos of the rainbow and brown trout has been plotted against the increase in their activity during larval development. It can be seen that although the level of activity is low at first in both species, once the larvae have become active for about 20% of the observation time further increases in activity are directly related to the rate of increase in size of the embryo.

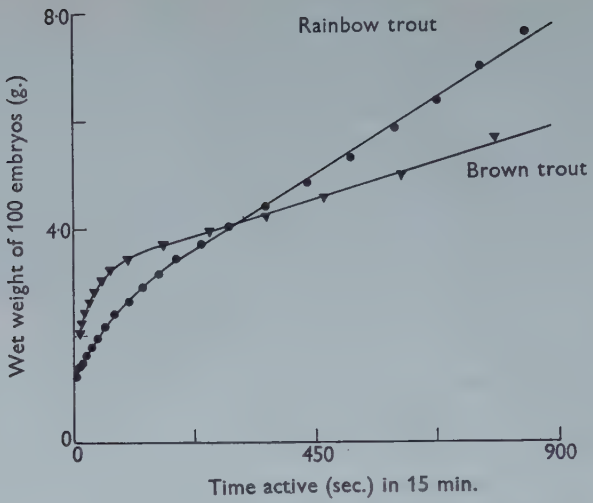

Fig. 7. Relationship of increase in activity at 4·6 m.c. during development, to the growth of the embryo in the rainbow trout and brown trout. The data for the growth of the embryos were derived by drawing a smooth curve through the values given by Gray (1928 *a*) and Smith (1952), for the wet weights of 100 trout embryos at different stages of development. ●, rainbow trout; ▼, brown trout.

The careful studies of Smith (1947, 1952) on the metabolism of the developing rainbow trout are of considerable interest in connexion with the present results. Smith describes a steady state of total heat production per gram weight of embryo, from the thirty-fifth day, when hatching occurred, until the sixtieth day of development. At about the sixtieth day there was a sudden burst of carbohydrate metabolism and of glyceride fat metabolism, whilst there was a sharp fall in the utilization of non-glyceride fat, which had been the main source of heat production since hatching. It is at precisely this time that the entire behaviour changes, and the trout larva assumes the behaviour of the trout fry. It seems probable that the changes in behaviour and metabolism are related. Smith (1952) suggests that the sudden metabolic changes denote the onset of starvation; he points out (personal communication) that although the yolk-sac still contains appreciable quantities of yolk (mostly glyceride fat with some carbohydrate), this is a protein starvation which will not be mitigated by the yolk reserves. Hunger has been shown to alter the reactions of adult fish to light (Woodhead, 1956). However, the changes observed in the trout larvae affect their entire behaviour and seem to be of a more profound nature than might be expected from hunger alone.

SUMMARY

1. The larvae of the brown trout, rainbow trout, and salmon were periodically active and negatively phototactic when illuminated. All three species exhibited a positive photo-orthokinesis, the duration of their activity being directly proportional to the log of the light intensity, over a limited range. The light threshold for the kinesis was about 0·005 m.c. but tended to be higher in many young larvae and

lower in larvae of a later stage. An upper limit, although more variable, was demonstrated in some individuals, and lay between about 10 and 100 m.c. in the older larvae.

2. It is suggested that a photo-orthokinesis, coupled with a negative phototaxis, serves well to maintain the larvae within the limits of their normal habitat at this stage of development.
3. No adaptation of the kinesis occurred after periods of up to 90 min. illumination. But larvae did exhibit a rhythm of activity, reaching peaks at intervals of about 15 min.
4. An increase in the general level of activity, at all light intensities, occurred as the larvae grew, until they were finally active throughout an observation period. At this point the behaviour of the young fish changed, in particular the negative phototaxis was lost and the fish soon began to feed.
5. The increase in the level of activity may be regarded as an index of development in trout and was shown to be related to the general growth of the embryo.
6. Rainbow trout ova hatch about a week earlier than brown trout at 10° C. This does not necessarily indicate a more rapid development in the rainbow trout, since the latter takes a week longer to achieve full activity, both species reaching this stage at about sixty days after fertilization. The behaviour of the salmon larva does not seem to be strictly comparable with that of the trout during the later stages of development.

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OSMOREGULATION IN SOME FRESHWATER PRAWNS

By GWYNETH PARRY
Senior Research Fellow, Ministry of Agriculture,
Fisheries and Food

(*Received* 9 *April* 1957)

I. INTRODUCTION

The different species of prawns of the family Palaemonidae are to be found in a range of environments from fresh water to marine, and some individual species are able to tolerate changes over almost the whole of this range. Two such species in Britain are *Palaemonetes varians* (Leach) which may live in very dilute brackish water, in saline marsh pools and in any salinity between these extremes; and *Palaemon longirostris* (H. Milne-Edwards) which may extend over a similar range. The distribution of these two species in Britain appears to stop just short of a completely freshwater environment (Gurney, 1923; Panikkar, 1941), but *P. longirostris* is reported from fresh water in Europe (Holthuis, 1950) and *P. varians* as varieties *macrogenitor* (Boas, 1898), *termajophilus* (Garbini, 1881; Seurat, 1922), or *lacustris* (Sollaud, 1923, 1933) is found in southern Europe. A recent review of the family (Holthuis, 1950) has accorded specific status to this southern European freshwater form as *Palaemonetes antennarius* (H. Milne-Edwards).

Osmoregulation in *Palaemonetes varians* has been studied by Panikkar (1941), but very little physiological information is available for *Palaemon longirostris* or *Palaemonetes antennarius*. Predictions of the osmoregulatory activity of the former were made by Gurney (1923), Schnakenbeck (1933) and Panikkar (1941), but there is no experimental work; while for the latter species there exists only a single record of the blood freezing-point made by Vialli (1925).

In this paper an account is given of some aspects of osmoregulation in *P. antennarius*, together with some comparative observations on *P. varians* and *Palaemon longirostris*.

II. METHODS

Palaemonetes antennarius was collected from two localities near Verona in North Italy: at Caldiero, a thermal spring in the Po valley, and at Peschiera where the River Mincio flows out from the Lake of Garda. The former source has water of some hardness and a temperature of 28° C. (Frederici, 1948) (*c.* 200 mg./l. $CaCO_3$, and appreciable amounts of magnesium and sulphate), while the latter has water of a lower mineral content (Marchesoni, 1952) (112 mg./l. $CaCO_3$).

P. varians in these experiments was collected from brackish farm drainage ditches near Breydon water (Norfolk), where the chlorinity varied from 0·38 to 1·01 ‰ Cl (10·8 to 28·4 mM./l. Cl). Many freshwater invertebrates were present in the same ditches.

Palaemon longirostris was obtained from the River Tamar (Devon) just downstream from the Calstock Bridge. The chlorinity at low water was 0·67‰ Cl (18·8 mM./l. Cl).

Measurements of the freezing-point depression were made with the apparatus described by Ramsay (1949) and Ramsay & Brown (1955). The freezing-point depression may be converted into terms of salt concentration by means of the relationship $\Delta/^{\circ}$ C. $\equiv 285$ mM./l. NaCl (Ramsay, 1949). Chloride was estimated in the blood and urine of *P. longirostris* by the method of Sendroy (Sendroy, 1937; van Slyke & Hiller, 1947), in which the chloride in solution is replaced by iodate and determined iodometrically. The remainder of the chloride measurements were made by a microdiffusion technique (Conway, 1951). Copenhagen sea water was used throughout as a standard.

The measurements of the rate of urine flow were made by a dye excretion method described in a previous paper (Parry, 1955).

III. RESULTS

Freezing-point depression of blood and urine

The freezing-point measurements of the blood and urine of the three species in fresh or slightly brackish water show that these two fluids are always approximately isosmotic, and are maintained at a level of concentration very much higher than that of their respective media (Table 1). The figures for *Palaemonetes varians* confirm the level of the mean for the blood of animals in a similar environment given by Panikkar (1941), who used a Hill–Baldes thermo-electric method. The measurements of blood and urine in *Palaemon longirostris* confirm that this species maintains the concentration of its body fluids at about the same level as *Palaemonetes varians*.

Table 1. *Freezing-point depression of blood and urine in prawns*

(No. of determinations in parentheses)

Species	Δ° C.		
	Blood	Urine	Medium
P. antennarius (Peschiera)	−0·75 ± 0·04 (6)	−0·67 ± 0·05 (10)	0·01
P. varians			
Parry	−1·05 (3)	−1·01 (3)	0·11
Panikkar	−1·26	Isotonic	0·10
P. longirostris	−0·95 (4)	−0·94 (4)	0·07

On the other hand the freezing-point depression of the blood of *P. antennarius* is of the same order as that of other freshwater decapods, e.g. *Cambarus clarkii* $\Delta = 0{\cdot}64^{\circ}$ C. (Lienemann, 1938) and *Astacus astacus* $\Delta = 0{\cdot}80^{\circ}$ C. (Schwabe, 1933), but it is lower than that found for *Eriocheir sinensis* in fresh water, $\Delta = 1{\cdot}18^{\circ}$ C. (Scholles, 1933), or for *Potamon edule* in fresh water, $\Delta = 1{\cdot}17^{\circ}$ C. (Duval, 1925), or of *Palaemonetes varians* or *Palaemon longirostris* in the lowest level of salinity they will tolerate. The urine of *Palaemonetes antennarius* is approximately isosmotic with the blood as it is in the brackish water prawns and in contrast to the dilute urine produced by the freshwater crayfishes.

Table 2. *Chloride content of blood and urine in prawns*

(No. of determinations in parentheses.)

Species	mM./l. chloride		
	Blood	Urine	Medium
P. antennarius (Peschiera)	120 ± 10 (15)	114 ± 15 (12)	0·25
P. antennarius (Caldiero)	109 ± 16 (12)	136 ± 14 (12)	0·85
P. antennarius (pooled)	116 ± 14 (27)	125 ± 12 (24)	—
P. varians	264 ± 36 (20)	314 ± 49 (19)	28
P. longirostris	301 ± 93 (6)	348 ± (40) 6	19

The chloride analyses (Table 2) show that in each of the three species the chloride concentration of the urine is of the same order as that of the blood, and they confirm the high salt content which was to be expected from the freezing-point measurements. However, the proportion of the total osmotic pressure contributed by chlorides varies between the different species. In *P. antennarius* the figures for chloride are low compared with those of the other two species, even when the lower value of the freezing-point depression is taken into account. If the concentrations of blood and urine are calculated solely in terms of sodium chloride, and the concentration of chloride calculated from this, it will be found that in *P. varians* and *Palaemon longirostris* the ratio Cl estimated : Cl measured is near to unity (0·85–1·20), while the ratio for *Palaemonetes antennarius* is only 0·56 for blood and 0·66 for urine. This suggests that chlorides are relatively less important than other constituents in maintaining the high osmotic level of the body fluids in this freshwater species.

The differences between the figures for chloride concentration in *P. antennarius* from the two environments are not statistically significant.

Rate of loss and gain of salts in Palaemonetes antennarius

Two sets of experiments were designed to measure the rate of loss of salts from *P. antennarius* when in distilled water. The first, using animals from both Peschiera and Caldiero, entailed measuring the conductivity of 200 ml. of water in which five animals (*c.* 0·4 g.) were kept. The water was changed hourly, and the quantity of chloride lost in these hourly intervals was calculated. The apparatus was calibrated with dilutions of standard sea water. Examples of these experiments are shown in Table 3. For animals from both environments the rate of loss of salts calculated from these experiments was 34·2 μM. NaCl/g./hr. (where the total salts lost are expressed as μM. NaCl).

If the same weight of animals (*c.* 0·4 g.) is put into 200 ml. of distilled water, and the medium is not changed, the animals lose salts at a decreasing rate. Prawns from Caldiero reach a steady state when the medium has a concentration of 0·99 mM./l. Cl (*c.* 24 hr.) after which the animals survive for many days. The prawns from Peschiera, on the other hand, show a similar decreasing rate of loss, but when the concentration of the medium was 0·42 mM./l. Cl the animals died (*c.* 10 hr.).

A second experiment to measure the rate of loss and gain of salts from the blood was made on animals from Peschiera. In this experiment, single animals (*c.* 0·1 g.) were kept in 100 ml. distilled water changed at 2-hourly intervals. Small samples of blood were taken for measurement of freezing-point. After 6 hr. the osmotic pressure of the blood had been appreciably reduced. On replacing these salt-depleted animals in their natural medium (Peschiera water) the blood concentration gradually regained its normal level in about 5 hr. (Table 4).

Table 3. *Total loss of salts by conductivity measurements in* Palaemonetes antennarius. $T = 18 \pm 1°C$.

Hours	Animals from Peschiera, 0·39 g. in 200 ml. distilled water		Animals from Caldiero, 0·38 g. in 200 ml. distilled water	
	Chloride ‰	Total mg. Cl lost	Chloride ‰	Total mg. Cl lost
0	0	0	0	0
1	0·00225	0·45	0·00215	0·43
2	0·00218	0·89	0·00245	0·92
3	0·00250	1·39	0·00229	1·38
4	0·00235	1·86	0·00247	1·87
5	0·00244	2·34	0·00225	2·32
6	0·00256	2·86	0·00262	2·85
7	0·00225	3·31	0·00203	3·25
8	0·00255	3·82	0·00245	3·74

Table 4. *Rate of loss and gain of salts from the blood of* Palaemonetes antennarius (*Peschiera*)

Hours	Δ° C. Blood	S.E.	n
0 hr. D.W.	0·749	±0·036	6
2 hr. D.W.	0·689	±0·061	7
4 hr. D.W.	0·660	±0·053	13
6 hr. D.W.	0·609	±0·045	7
2 hr. P.W.	0·677	±0·013	4
3 hr. P.W.	0·727	±0·088	3
5 hr. P.W.	0·740	±0·045	10

D.W. = distilled water; P.W. = Peschiera water. n = no. of determinations.

In order to calculate from this data the rates of loss and gain of salts it will be assumed that the blood volume in ml. is one-third of the body weight in g. This gives: loss, 2·21 μM./g./hr.; gain, 2·56 μM./g./hr.

Rate of urine flow in Palaemonetes antennarius

The rate of urine flow was determined by injecting indigo-carmine into the haemocoele and measuring the rate of its elimination. Prawns from Peschiera and Caldiero were used, and a slightly different rate of flow was measured for each, but this difference does not appear to be significant. These rates may be compared with that previously found for *P. varians* in a dilute medium and the one measurement (obtained by direct cannulation of the gland) for *Palaemon longirostris* (Table 5). In a previous paper (Parry, 1955) it was estimated that if *Palaemonetes varians* was to live in completely fresh water its urine flow would be about 2% body weight/hr. The rates measured for both Peschiera and Caldiero animals are very close to this.

Table 5. *Rate of urine flow estimated by dye excretion in prawns*

(No. of determinations in parentheses.)

Species	Rate, as % body wt./hr..	Medium
P. antennarius (Peschiera)	2·51 ± 1·06 (57)	F.W.
P. antennarius (Caldiero)	1·89 ± 0·62 (49)	F.W.
P. varians	1·63 ± 0·09 (34)	5–10% S.W.
P. longirostris	1·82 (direct cannulation)	7% S.W.

This apparently high rate of urine flow in these prawns is in contrast to the rate reported for other freshwater decapods. In *Cambarus* and *Astacus* the rates are only one-tenth of this (Scholles, 1933; Lienemann, 1938). In *Eriocheir* and *Potamon*, which like *Palaemonetes* cannot produce a hypo-osmotic urine, the urine flow is said to be very low (Scholles, 1933; Schlieper & Herrmann, 1940). It is clear that while *Palaemonetes* may have saved some osmotic energy in the reduction of the blood concentration (Potts, 1955) it is losing salts constantly in the excretion from the antennal glands.

IV. CONCLUSIONS

Palaemonetes antennarius has often been described as a variety of *P. varians* (Garbini, 1881; Boas, 1889; Seurat, 1922; Sollaud, 1923), but more recently has been given the status of a separate species, although Holthuis (1950), in speaking of this and some other species, is uncertain (on morphological and embryological grounds) whether they can be maintained as separate species. Measurements of the freezing-point depression and chloride concentration of blood and urine of *P. antennarius* indicate that this animal is distinct (on physiological grounds) from *P. varians*.

Although of limited distribution *P. antennarius* is not a rare animal and must be regarded as a successful inhabitant of fresh water. Unlike *P. varians*, it can live in completely fresh and relatively soft water; unlike *Palaemon longirostris* it does not return to salt waters, for breeding. At the same time, however, it lacks those adaptations to a freshwater environment which have been achieved by the freshwater crayfishes, for while its blood concentration is as low as that of many other freshwater inhabitants, it continues to produce an isosmotic urine in a copious flow. Scholles (1933) failed to find any modification in the size or structure of the antennal glands, compared with those of the brackish *Palaemonetes varians*, in contrast to the enlarged glands found in the freshwater crayfishes or gammarids.

Experiments designed to show the rate of loss of salts from the blood in distilled water and of gain in a very dilute natural water show that loss and gain are of the same order, and there is little doubt that under natural conditions there is a constant absorption of ions to compensate the osmotic inflow of water and the loss of salts through the antennal glands.

P. antennarius thus appears to be a successful invader of fresh water, but one which is incompletely adapted to this environment, presumably at considerable energetic expense. While distinct from its near relative *P. varians*, it has changed relatively little physiologically or morphologically. If it is as geographically isolated as it appears, it would be an interesting evolutionary study to await the development of further distinctions. Comparison of the two local populations at Caldiero and at Peschiera gave indications of small differences in osmotic behaviour, but it was not possible to show that these differences were statistically significant.

V. SUMMARY

1. A freshwater prawn, *Palaemonetes antennarius*, from Italy, is shown to have a different level of salt concentration in the blood and urine compared with that of the brackish water *P. varians* and *Palaemon longirostris* in Britain.

2. Although this prawn lives in fresh water the urine is isosmotic with the blood and is produced at a rate of about 2% body weight/hr.

3. An uptake of salts from a very dilute medium has been demonstrated in salt-depleted animals.

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